Review

PU-H71: An improvement on nature’s solutions to oncogenic Hsp90 addiction

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IPI-504 (retaspimycin)
STA-9090 (ganetespib)
PU3 (9-butyl-8(3,4,5-trimethoxy-benzyl)-9H-purin-6-ylamine)
BIIB021 (6-chloro-9-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-9H-purin-2-amine)
PU-H71 (8-[(6-iodo-1,3-benzodioxol-5-yl)sulfanyl]-9-[3-(propan-2-ylamino)propyl]purin-6-amine)

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ABSTRACT

Despite recent advances in precision medicine, many molecular-based antineoplastic agents do not potentiate sustainable long term remissions, warranting the investigation of novel therapeutic strategies. Heat shock protein 90 (Hsp90) is a molecular chaperone that not only has oncogenic properties, but also has distinct expression profiles in malignant and normal cells, providing a rational strategy to attain preferential damage. Prior attempts to target Hsp90 with natural product-based compounds have been hampered by their associated off target toxicities, suggesting that novel, fully synthetic inhibitors may be required to achieve the specificity necessary for therapeutic efficacy. Therefore, this review highlights the antineoplastic potential of PU-H71 (8-[(6-iodo-1,3-benzodioxol-5-yl)sulfanyl]-9-[3-(propan-2-ylamino)propyl]purin-6-amine), a novel purine based analog that has shown efficacy in many preclinical models of malignancy, and is now under clinical examination. In addition, the review suggests potential concomitant therapeutic approaches that may be particularly beneficial to molecular-based, as well as traditional cytotoxic cancer chemotherapy.

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1. Introduction

The world of antineoplastic drug discovery is changing. Traditionally, agents designed for cancer chemotherapy rely on the principal that malignant cells have higher proliferation rates than normal cells, enabling the selective poisoning of malignant tissue with agents that disrupt vital components of the cell cycle. Specifically, these traditional antineoplastic agents target either nucleic acids and proteins involved in DNA synthesis and transcription, or microtubules responsible for chromosome congregation and proper segregation of chromosomes during anaphase [1]. While effective against several hematological malignancies and solid tumors, traditional chemotherapeutic agents are inherently limited by their less than ideal toxicity profiles, producing significant deleterious off target effects in patients that limit dosing schedules. Further, many cancers are refractory or eventually develop resistance to traditional cytotoxic agents, thereby potentiating drug resistant tumors that portend a very poor prognosis.

In recent years, the commendable progress in molecular biology has enabled investigators to probe deregulated signaling pathways and oncogenes that are imperative for carcinogenesis. This has launched the introduction of precision medicine in cancer chemotherapy in which clinicians now have the capability of selecting optimal therapies based on the genetic and phenotypic profile of the patient’s malignancy in addition to traditional broad spanning cytotoxic antineoplastic intervention. Novel targeted approaches, such as hormonal therapy, monoclonal antibodies (mABs) and associated immunocojugates, and small molecule kinase inhibitors have given clinicians an unprecedented ability to discriminate between neoplastic and normal tissue. Nevertheless, these agents suffer from many of the same pitfalls associated with traditional cytotoxic chemotherapy. Hormonal therapy is limited in the scope of tumors perturbed by such intervention, and such protocols have a low, but notable risk of potentiating other malignancies in addition to inducing an initial surge in tumor growth [2,3]. In the case of immunotherapy, tumors are not homogenous in their cell surface protein expression, and it is possible that some cells lack the required epitope, or inadvertently shed the required antigen if it is unnecessary for cell survival [4-7]. Kinase inhibitors enable the clinician to select for patient malignancies that rely on specific aberrantly expressed pathways, but are often toxic to normal cells based on the requirement of these signals for homeostatic functions [8,9].

Although the amount of progress cancer chemotherapy has made in recent years is noteworthy, there is an apparent need for chemotherapeutic agents that work by mechanisms not currently approved for clinical use. Recent work has implicated molecular chaperones, ubiquitously expressed proteins vital to the formation of certain macromolecular structures, to be a particularly intriguing target of interest. Molecular chaperones are members of a unique class of proteins that constitute the chaperone, an interconnected network of molecular chaperones, co-chaperones, and folding enzymes encoded by as many as 169 genes that help maintain cell stability by regulating proteome machinery required for a vast array of housekeeping activities [10]. The ubiquitous nature of chaperones initially suggested that the chaperone was of little interest to oncology as such proteins are highly expressed in both normal and malignant cells. However, there exist inherent differences in the expression profile of chaperones under normal conditions and those induced by oncogenic stress [10-12], enabling the targeted inhibition of proteins vital for malignant tissue.

A particular molecular chaperone of interest is heat shock protein 90 (Hsp90), which has been shown to support aberrant expression of key oncoproteins such as ALK (anaplastic lymphoma kinase), BCR-ABL (break point cluster-Abelson tyrosine kinase) BRAF (serine/threonine-protein kinase B-Raf), CDK4 (cyclin-dependent kinase 4), CRAF (serine/threonine-protein kinase C-Raf), HER2 (human epidermal growth factor receptor 2), JAK2 (Janus kinase 2), KIT (Mast/steal cell growth factor receptor, proto-oncogene c-Kit) MET (mesenchymal epithelial transition factor), and STK33 (serine/threonine kinase 33) [13-17]. Initial attempts to target Hsp90 with inhibitors that resemble the structure of the natural products geldanamycin and radicicol indicated that successful targeting of the chaperone could yield therapeutic benefit at the clinical level, but such compounds are plagued by unfavorable toxicity profiles that limit achievable doses [18,19]. Consequently, these lower concentrations are unable to sustain consistent antineoplastic activity, and have limited clinical utility. Nevertheless, structure–activity studies have enabled the design of novel purine-based inhibitors [20-23] that have demonstrated notable specificity toward oncogenic Hsp90. The most promising of these agents, PU-H71, is currently under clinical investigation, and will be discussed at length to demonstrate how rational drug design can potentiate the development of chemotherapeutic agents that are highly potent and selective toward malignant tissue.

2. Structure and function of heat shock protein 90

Hsp90 refers to a subgroup of molecular chaperones that fold client proteins to their active conformation through their ATPase activity, and have a characteristic Bergerat fold (unique structural ATP-binding domain) near the N-terminus. There are four different paralogues of Hsp90, each of which has a distinct cellular location; Hsp90α and Hsp90β in the cytoplasm, gp96 (Grp96, Erp99, or endoplasmin) in the endoplasmic reticulum, and Trap-1 in the mitochondria [22,23]. Hsp90 substrates are regulated by the N-terminal ATPase domain, which binds and hydrolyses ATP to mediate association/dissociation cycles between Hsp90 and client proteins [24,25]. This ATPase activity produces several conformations of Hsp90 based on ATP binding status and whether the nucleotide has been hydrolyzed (Fig. 1). The activity of Hsp90 is further regulated by co-chaperones which aid in the conversion
between ATP- and ADP-bound states and modulate the formation of client-specific complexes. Specifically, its 70 kDa co-chaperone Hsp70 initiates the association of the client protein with Hsp90 through a bridging protein called Hsp-organizing protein (HOP) (Fig. 1) [26–28]. Together, these proteins ensure proper folding of essential proteins vital for both normal and carcinogenic processes.

Traditionally, Hsp90 inhibitors work by binding the ATP domain of the protein, thereby preventing proper folding of client proteins. Since many oncoproteins are also clients of Hsp90 [13–17], these macromolecules are targeted for destruction, often via the ubiquitin/proteasome system [12]. Therefore, malignant growths are starved of proteins vital for maintaining the malignant phenotype, eventually initiating apoptotic mechanisms. Although Hsp90 is ubiquitously expressed in both normal and neoplastic cells, potential therapeutic intervention is possible due to its distinctive activation in normal and stressed environments. As demonstrated in multiple malignant cell lines, Hsp90 typically forms complexes with co-chaperones that have a high affinity for ATP/ADP and other ligands of this binding pocket (including ATPase inhibitors), whereas in normal tissues, Hsp90 exists primarily in an uncomplexed, low affinity state [29,30]. The shift in equilibrium between latent and activated states is thought to be dictated by the amount of stresses incurred by the cell, which is increased in transformed counterparts due to the effects of mutated and deregulated proteins, hypoxia, and a low nutrient concentration environment [21].

In addition, it has been elucidated that cancer cells do not have a uniform composition of high affinity Hsp90. Rather, the proteins are a composite of housekeeping Hsp90s (with low affinity to particular small molecule inhibitors that are very similar to Hsp90 found in normal cells) and onogenic Hsp90s [31]. These epigenetically distinct Hsp90 forms are similar to the ones used during cellular stress, but are enriched or expanded in cancer cells. Consequently, malignancies use the elevated levels of stress Hsp90s to maintain altered proteins and protein expression levels vital for cancer pathology [31]. The therapeutic activity of Hsp90 inhibitors lies in their ability to preferentially interact with onogenic Hsp90s, acting on housekeeping Hsp90s only at higher or at saturating concentrations [10]. To summarize, neoplastic cells contain a complex mixture of Hsp90s. A majority of these chaperones perform housekeeping functions similarly to non-stressed, normal cells, but a notable fraction buffers the proteome altered in the process of oncogenic stress. These stress-induced and stress-associated Hsp90s are proposed to be epigenetically and thermodynamically distinct from housekeeping Hsp90s, thereby providing a rationale for their selective targeting by small molecule inhibitors.

3. Initial attempts to target heat shock protein 90 using semisynthetic derivatives of natural products

Although Hsp90 is a relatively novel therapeutic target in oncology, there have already been numerous clinical studies evaluating the potential of semisynthetic derivatives based on the structure of natural products geldanamycin and radicicol. Geldanamycin (Fig. 2) is a benzoquinone ansamycin antibiotic originally isolated in 1970.
Fig. 2. Natural product Hsp90 inhibitors. Important functional moieties are highlighted with color coded outlines; blue (benzoquinone moiety), red (carbamate moiety), and green (resorcinol moiety). The numbering system for each compound is highlighted in blue. Molecular weights for geldanamycin and radicicol are indicated underneath their corresponding molecular structures. (For interpretation of the references to color in this legend, the reader is referred to the web version of the article.)

from Streptomyces hygroscopicus [32], the same bacterium that produces the mTOR (mechanistic target of rapamycin) inhibitor rapamycin. Geldanamycin binds the ATP binding pocket of Hsp90 by positioning the macrocyclic ansa ring and carbamate moiety toward the bottom of the pocket, while the benzoquinone ring is oriented toward the top of the pocket [33]. Once appropriately positioned, geldanamycin forms hydrogen bonds within the ATP binding pocket, including interactions between the macrocycle carbamate carbonyl and a key aspartate residue at the bottom of the pocket, thereby mimicking the actions of ATP [33]. While this activity potently inhibits Hsp90 function, the antibiotic was never investigated at the clinical level due to its poor in vivo stability and toxicity stemming from the benzoquinone moiety that undergoes reductive metabolism by nicotinamide adenine dinucleotide phosphate (NADPH): quinone oxidoreductase (NQ01) before it acts against Hsp90 [34–36].

Nevertheless, geldanamycin provided an important scaffold to base semisynthetic derivatives with improved solubility and a reduced toxicity profile that could be assessed at the clinical level. Replacement of the non-essential C-17 methoxy group of geldanamycin via substitution with various amines provided many semisynthetic derivatives, one of which was 17-AAG (17-N-allylamino-17-demethoxygeldanamycin/tanespimycin), the first Hsp90 inhibitor to be clinically evaluated. 17-AAG retains the anti-Hsp90 activity observed with its natural product counterpart, but has an improved toxicity profile. Nevertheless, 17-AAG produced disappointing clinical results, as the agent still had an unacceptable pharmaceutical and toxicity profile, even after the development of an improved formulation [37,38]. In addition, the therapeutic index of 17-AAG is fundamentally limited by the benzoquinone moiety that causes marked hepatotoxicity and potentially may constitute a mechanism of drug resistance in patients with a mutation in NQ01, the enzyme that acts as its anticancer activity [39].

Despite these shortcomings, investigators have pressed on with the development of geldanamycin-based Hsp90 inhibitors, leading to the development of 17-DMAG (17-desmethoxy-17-N,N-dimethylaminoethylaminoacetamidogeldanamycin/alvespimycin). To improve aqueous solubility, 17-DMAG contains an ionizable N,N-dimethylethylamine group instead of a methoxy group at C-17. This slight alteration tremendously improves its hydrophilicity and oral bioavailability [40,41], while still retaining equal, if not superior, antitumor activity in comparison to 17-AAG [39]. Although 17-DMAG still potentiates notable toxicity in patients (liver, lung, ocular and cardiac toxicities in addition to common side effects such as diarrhea, fatigue and nausea [42–45]) the agent does appear to be tolerable, and has shown increased activity when combined with the HER2-targeting mAB trastuzumab [46]. In addition to 17-DMAG, IPI-504 (retaspimycin) is another geldanamycin-based Hsp90 inhibitor that has shown potential at the clinical level. IPI-504 is a reduced quinone form of 17-AAG, and has higher solubility than either 17-AAG or 17-DMAG [39]. Further, since IPI-504 lacks the benzoquinone moiety, it does not potentiate hepatotoxicity as much as its related congeners [39]. IPI-504 has shown notable activity against EML4-ALK (echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase) positive non-small cell lung carcinoma (NSCLC) [46,47], and HER2 positive breast carcinoma [48,49].

Radicicol-based Hsp90 inhibitors have also been evaluated at the clinical level. Initially isolated in 1953 from the fungus Monocilium nordinii [50], radicicol (Fig. 2) is a 14-member macrolide that demonstrates potent Hsp90 activity due to a resorcinol moiety, which binds the same aspartate residue as geldanamycin via its 3-hydroxyl group. However, radicicol is also associated with the same issues of low solubility and therapeutic index in addition to being unstable in plasma [39,51], thereby requiring the intervention of medicinal chemistry. Unlike geldanamycin-based compounds, those that employ the resorcinol moiety are highly heterogeneous in structure, and are only loosely related by their pharmacophore.

The resorcinol core of radicicol is found in a number of agents that have been clinically evaluated, including NVP-AUY922, AT-13387, STA-9090 (ganetespib), and KW-2478. Out of these agents,
STA-9090 has so far made the most progress in research and development, being evaluated in over 25 clinical studies, including an ongoing Phase III trial [39]. STA-9090 is a novel resorcinolic triazolone Hsp90 inhibitor that potently inhibits cell proliferation and induces apoptosis in a variety of cancer cell lines resistant to tyrosine kinase inhibitors (TKIs) and 17-AAG in vitro and in vivo [52,17,53]. The agent has notable, but likely manageable side effects including diarrhea, hyperbilirubinemia/hyponatremia, QTc prolongation, and elevated transaminases, a potential indicator of liver damage [54–57]. STA-9090 appears to show clinical efficacy against malignancies positive for known Hsp90 clients, including EML4-ALK positive NSCLC [58], HER2 positive breast carcinoma [59], and KIT positive gastrointestinal stromal tumor (GIST) [60,61]. Since Hsp90 inhibition is known to induce G2/M arrest [61,62], STA-9090 has been used in combination with mitotic inhibitors at the preclinical and clinical level, with enhanced cytotoxicity being observed [39,63–65].

4. Designing a rational inhibitor of heat shock protein 90 to improve upon nature’s solutions

Although there is a considerable diversity of Hsp90 inhibitors that have been, or are currently being clinically evaluated, none of these drug candidates are anywhere near approval by the U.S. Food and Drug Administration (FDA), or other pharmaceutical regulating bodies. While some of the more recent Hsp90 inhibitors show some clinical potential, the original agents of this drug class suffered from notable off target effects, limiting the concentrations that could safely be administered in patients. Many of these agents are natural product–based compounds that happen to potently inhibit Hsp90 function, but were not specifically designed for inhibiting the oncogenic functions of the protein. Therefore, medicinal chemists have sought to design fully synthetic Hsp90 inhibitors that retain or exceed the activity of natural products, but demonstrate higher specificity, greatly increasing the therapeutic index. One such drug class, purine-based Hsp90 inhibitors, was discovered by Chiosis and colleagues at the Memorial Sloan Kettering Cancer Center (New York, NY, USA). As will be discussed, the most promising of these fully synthetic inhibitors, PU3, is potently cytotoxic against vital oncoproteins, including those not previously characterized as Hsp90 clients, while demonstrating notable preferential activity against malignant tissue.


Around the turn of this century, it was apparent that Hsp90 was a potential antineoplastic target that was in desperate need of inhibitors with improved therapeutic indices. Therefore, Chiosis and colleagues set about designing novel compounds through structure–activity rationale. To synthesize compounds specific to Hsp90, Chiosis et al. meticulously examined the Hsp90 ATP binding pocket, and realized that an agent would have to satisfy several hydrophilic and lipophilic interactions at this site, while retaining higher affinity for the pocket than ADP [20]. The agent has notable, but likely manageable side effects including diarrhea, hyperbilirubinemia/hyponatremia, QTc prolongation, and elevated transaminases, a potential indicator of liver damage [54–57]. STA-9090 appears to show clinical efficacy against malignancies positive for known Hsp90 clients, including EML4-ALK positive NSCLC [58], HER2 positive breast carcinoma [59], and KIT positive gastrointestinal stromal tumor (GIST) [60,61]. Since Hsp90 inhibition is known to induce G2/M arrest [61,62], STA-9090 has been used in combination with mitotic inhibitors at the preclinical and clinical level, with enhanced cytotoxicity being observed [39,63–65].

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By understanding the differences between the ATP pocket of Hsp90, and homologous pockets in other proteins, Chiosis et al. designed PU3 (9-(butyl-8’3,4,5-trimethoxy-benzyl)-9H-purin-6-ylamine; Fig. 3A), the first fully synthetic pan-Hsp90 inhibitor [20]. Although PU3 was shown to preferentially damage breast carcinoma cells by inhibiting oncogenic Hsp90 function and even inducing differentiation in MCF7 human breast carcinoma cells, PU3 was not as potent as 17-AAG. Nevertheless, the study demonstrated that fully synthetic Hsp90 inhibitors could be rationally developed, suggesting that the activity of purine-based compounds could be improved through appropriate structural alterations. Later structure–activity relationship analyses revealed that PU3 conforms to the ADP binding site of Hsp90α and β, with residues 104–111 adopting a helical conformation to provide space for the trimethoxy phenyl moiety to bind. Further, the purine ring mimics the adenine of ADP/ATP, occupying the same position as is seen with the nucleotide [66]. Chiosis and colleagues later went on to optimize this novel class of compounds, resulting in congeners bearing a thiouether bridge to connect the purine nucleus to substituted phenyl rings. These new compounds retain the primary amine attached to the purine core and contain an aryl moiety separated by ~5 Å to augment binding to Hsp90 [18]. Of note, PU-H58 (8-[(6-bromo-1,3-benzodioxol-5-yl)sulfanyl]-9-(4-pentyn-1-yl)-9H-purin-6-amine) demonstrated much higher potency than the original compound, and PU24F-CI (2-fluoro-pent-4-ynyl-8-(2-chloro-3,4,5-trimethoxy-benzyl)-9H-purin-6-ylamine) had an affinity for the N-terminus of Hsp90 that was 30 times greater than PU3 [67,68].

In addition, Biogen idec demonstrated that the presence of an amino group on the C-2 position of the purine nucleus enables multiple possibilities for hydrogen bonding, thereby allowing an aromatic group to be attached at N-9 rather than the C-8 of the purine. This structural modification resulted in the production of BIB021 (6-chloro-9-[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]-9H-purin-2-amine), which entered clinical trials in 2005 [18]. BIB021 has several associated off target effects, including dose limiting toxicities of syncope and dizziness, and other grade 3 or 4 toxicities such as fatigue, hyponatremia and hypoglycemia. Nevertheless, the agent does appear to elicit therapeutically relevant anticancer activity at the clinical level, and is still under evaluation [69,70].

5. PU-H71 transcends prior attempts to optimize purine-based heat shock protein 90 inhibitors

Although Chiosis and colleagues potentiated the development of completely synthetic Hsp90 inhibitors, it was not until the synthesis of PU-H71 (8-[(5-iodo-1,3-benzodioxol-5-yl)sulfanyl]-9-[3-(propan-2-ylamino)propyl]purin-6-amine; Fig. 3A) that the research group fully established the potential of purine-based compounds. Due to its iodide functional group, the positron emission tomography (PET) radionuclide 124I can be inserted to produce the imaging agent 124I-PU-H71. Conveniently, the PET agent is identical to PU-H71 in regards to activity, and its physical half-life of 4.02 days allows serial imaging for monitoring tumor PU-H71 concentrations for multiple days [18]. PU-H71 was initially reported in a structure–activity relationship screening of multiple derivatives designed to examine the effects of various substituents on anti-Hsp90 activity [21]. Out of this comprehensive screening, PU-H71 demonstrated potent antineoplastic effects, while demonstrating remarkable specificity toward transformed cells; binding affinity for Hsp90 from normal heart and lung tissues was 2-logs lower than Hsp90 from SKBr3 human breast adenocarcinoma cells. This
Fig. 3. Purine-based Hsp90 inhibitors. (A) Structures and molecular weights of PU3 and PU-H71. (B) PU-H71 differentiates between normal and oncogenic Hsp90. Although PU-H71 has high affinity for oncogenic Hsp90 at low concentrations, the agent needs to be administered at high to saturating concentrations to have marked inhibitory activity against normal Hsp90. These observations form the rationale for administering PU-H71 at concentrations that will preferentially inhibit oncogenic Hsp90 used by neoplastic cells to stabilize oncoprotein clients.

Specificity translated into over 50-fold selectivity in inhibiting the growth of malignant cells in comparison to normal fibroblasts. Further, no significant cell death was observed in PU-H71 treated MRC-5 human lung fibroblasts even at the highest tested concentrations. The preferential activity of PU-H71 against cancerous tissue under concentrations that do not perturb normal cells has been confirmed in multiple cell types (Fig. 3B) [71–73]. Since this initial study, PU-H71 has been shown to be highly effective in vitro and in vivo against several malignancies, each of which will be briefly highlighted to exemplify the potential PU-H71 has in cancer chemotherapy.

5.1. Breast carcinoma

It has been long established that HER2/Neu overexpressing breast carcinoma often relies on Hsp90, as the molecular chaperone stabilizes its client oncoprotein, thereby maintaining a large pool of active and folded oncoproteins that potentiate the neoplastic phenotype [74–77]. Consequently, many of the Hsp90 inhibitors previously mentioned have been examined at the clinical level as a novel chemotherapeutic intervention for HER2 positive breast carcinoma, with variable results being attained [78,44,79,80]. In the initial study of PU-H71, the agent demonstrated a very high propensity to initiate the degradation of HER2, and subsequently was notably cytotoxic against malignant, but not normal cells [21]. This alone made PU-H71 an exciting pharmaceutical prospect that warranted further preclinical investigation. What made PU-H71 distinct from previous Hsp90 inhibitors is that it also displayed potent in vitro and in vivo activity against triple negative breast carcinoma (TNBC), a malignancy that does not express the genes for estrogen receptor, progesterone receptor, or HER2/Neu.
In previous clinical studies, 17-AAG, IPI-504, and 17-DMAG were all shown to be ineffective against TNBC tumors [81,82], suggesting that Hsp90 inhibitors may have less relevance against this subtype of breast carcinoma. Although PU-H71 has not been formally evaluated against TNBC at the clinical level, the agent has notable preclinical antineoplastic activity against this subtype of breast carcinoma that may warrant examination in afflicted patients. The agent demonstrated that multiple human TNBC cell lines (MDA-MB-468, MDA-MB-231, and HCC-1806) were markedly perturbed by Hsp90 inhibition [83]. Further, multiple 75 mg/kg i.p. doses of PU-H71 induced complete responses and tumor regressions in TNBC xenografts without toxicity to the host, allowing TNBC tumors to be treated with PU-H71 over several cycles that extended over 5 months. By contrast, 20 or 30 mg/kg of 17-DMAG administered i.p. daily for 3 days to tumor-bearing mice induced significant weight loss, diarrhea, and a high incidence of drug associated deaths (23% of the population), in addition to being less efficacious in regards to antitumor activity [41,84]. More importantly, PU-H71 treatments appeared to have sustainable antitumor activity, as no distinct evidence of resistance or host toxicity were observed over this time frame [83]. This potent activity was attributed to PU-H71-mediated downregulation of an assortment of known oncoproteins, including EGFR, IGF1R (insulin-like growth factor 1 receptor), HER3, c-KIT, and Raf-1 in addition to MAPK/ERK (MAPK; mitogen-activated protein kinase was originally called ERK; extracellular signal-regulated kinase) pathway components p-ERK2 and p90RSK that had not previously been reported as Hsp90 clients. In addition, PU-H71 induced G2/M arrest in the TNBC cells, attributed to a notable reduction in CDK1 (cyclin-dependent kinase 1) and Chk1 (checkpoint kinase 1), and PU-H71 potentiated apoptosis through Akt (also referred to as PKB; protein kinase B) and Bcl-xl downregulation.

Interestingly, it also appeared that PU-H71 may have exerted antineoplastic activity by decreasing the metastatic potential of TNBC cells [83]. Although TNBC is not dependent on estrogen receptor, progesterone receptor or HER2 for maintaining malignant characteristics, many cases of this subset of breast carcinoma have elevated levels of nuclear factor κB (NF-κB), potentiating enhanced cell survival by suppressing apoptosis, induction of epithelial–mesenchymal transitions (EMT), resistance to chemotherapy, and the invasive and metastatic propensity of these tumors [85–87]. PU-H71 was shown to be in complex with several critical components of the NF-κB pathway, including IRAK-1 (interleukin-1 receptor-associated kinase 1), Tab2/3 (TAK1-binding protein 2 and 3), and TBK1 (NAK/NF-κB-activated kinase). These interactions with the NF-κB pathway are potentially vital for exerting antitumor activity, as the IRAK1/Tab complex activates TAK1, which directly phosphorylates IKKβ at the activation loop to activate the IKK complex, resulting in NF-κB activation [88,89]. By inducing proteasome-mediated degradation of IRAK-1 and TBK1, 0.5 and 1 μM PU-H71 reduced NF-κB activity in MDA-MB-231 human breast carcinoma cells by ~84% and 90% respectively. Further, administration of PU-H71 substantially reduced Akt and ERK levels, which are both known to promote the metastatic phenotype. By contrast, administration of geldanamycin or 17-AAG transiently promotes Akt and ERK activation in breast carcinoma cells, an observation that has attributed these Hsp90 inhibitors to potentially increasing the metastatic potential of certain malignancies [90]. More importantly, PU-H71 demonstrated IC50 values 2–8 fold lower than 17-AAG against all examined TNBC cell lines, indicative of higher in vitro cytotoxicity. Taken together, it appears that PU-H71 is likely superior to geldanamycin-based Hsp90 inhibitors in the preclinical treatment of TNBC, indicating that the agent may have clinical potential against this aggressive breast carcinoma subtype.

### 5.2. Hematological malignancies

Hematological malignancies constitute a diverse constellation of diseases linked by the transformation of normal blood components into aberrantly proliferating neoplasms. Consequently, these malignancies are characterized by a diverse assortment of aberrant signaling cascades and oncoproteins. Despite this diversity, PU-H71 appears to be effective against many of these diseases, with efficacy being demonstrated against myeloproliferative neoplasms (MPNs), lymphomas, and multiple myeloma.

### 5.3. Myeloproliferative neoplasms

MPNs refer to clonal hematological malignancies of the myeloid lineage that are characterized by an elevated number of blood cells, but lack many of the features of fully transformed cells, and have yet to progress into a more aggressive stage, such as myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) [91,92]. These blood disorders include chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocytosis (ET), and primary myelofibrosis (PMF). MPNs typically demonstrate characteristic driver mutations, the two most notable being the BCR-ABL fusion protein that constitutively activates the ABL1 tyrosine kinase, and somatic activating mutations in JAK2 [31]. Interestingly, PU-H71 demonstrates high affinity for Hsp90s in K562 human CML cells that contain the oncogenic BCR-ABL fusion protein, but not the wild type ABL tyrosine kinase [31]. However, the Hsp90-targeting mAB H9010 indiscriminately binds Hsp90, regardless of whether it contains BCR-ABL or ABL1. These data suggest the existence of distinct pools of Hsp90 that are preferentially bound to either BCR-ABL or ABL1 in CML cells [31]. It also suggests that PU-H71 can specifically target Hsp90s engaged in oncogenic activity, an observation with basic research and clinical implications. By contrast, synthetic Hsp90 inhibitors SNX-2112 and NVP-AUY922, as well as geldanamycin were less effective at isolating Hsp90s bound to BCR-ABL, with NVP-AUY922 showing activity very similar to H9010. The study also indicated that PU-H71 could be used as a molecular probe to uncover novel carcinogenic mechanisms in CML and potentially other neoplasms, as the agent was used in combination with affinity based proteomics to uncover a new STAT activation mechanism that was not previously characterized [31]. Future studies will hopefully produce novel targets and/or concomitant chemotherapeutic strategies to increase the overall efficacy of various treatment protocols.

Although PU-H71 may have potential as a chemotherapeutic agent against BCR-ABL driven hematological malignancies (Philadelphia chromosome positive CML and Philadelphia chromosome positive acute lymphoid leukemia; ALL), TKIs have already demonstrated commendable efficacy against such neoplasms. Therefore, PU-H71 may have greater utility against MPNs that are characterized by Janus kinase-signal transducer and activator of transcription (JAK-STAT) driver mutations, which have been less successfully targeted by JAK inhibitors. As demonstrated in numerous studies, tumorigenic versions of JAK2 appear to be a faithful client of Hsp90 [14,92–95], suggesting that PU-H71 may be a novel therapeutic modality by which to disrupt oncogenic JAK-STAT signaling. Indeed, it has been demonstrated that the Hsp90 inhibitor potentiates a lower IC50 value against cell lines bearing mutations that result in constitutive activation of the JAK-STAT signaling pathway (Jak2V617F and MPLW515L) in comparison to Philadelphia chromosome positive cell lines (Ba/F3 murine interleukin-3 dependent pro–B and KU812 chronic myeloid leukemia cells) and JAK2 wild type THP1 human acute monocytic leukemia cells [96]. Further, PU-H71 appears to preferentially perturb neoplastic cell signaling, as the agent potently inhibited STAT5 phosphorylation in MPN patient samples, but not cord blood.
samples, consistent with JAK2-dependent signaling by MPN cells [86]. In addition, PU-H71 inhibited mutant-associated erythrocytosis and megakaryopoiesis in JAK2V617F and MPLW515L murine models, without affecting normal erythrocytosis and megakaryopoiesis. Peritoneal tumors were consistently highly susceptible to PU-H71 [111,112]. In vivo, PU-H71 significantly prolonged the survival of Bcl6-dependent tumor-bearing mice, but not Bcl6-independent DLBCL. Toledo xenografts at comparable doses [111]. Important for potential clinical applications, PU-H71 was preferentially retained in DLBCLs compared to normal tissues at pharmacologically relevant concentrations at all timepoints examined. Further, PU-H71 facilitated the upregulation of Bcl6 target genes involved in DNA damage checkpoints [113], suggesting that the agent may sensitize certain DLBCL tumors to the cytotoxic effects of chemotherapeutic agents currently used at the clinical level. This potential therapeutic strategy may be supplemented with RI-BPI, a retro-inverted Bcl6 peptide inhibitor that induces the expression of p300 (p300 lysine acetyltransferase) and BAT3, which potentiate cell death in DLBCL cell lines [113]. Concomitant administration of RI-BPI and PU-H71 was remarkably potent against DLBCL xenografts, with 50% of mice treated with both agents having no evidence of tumor when their matched controls were sacrificed due to complications from the tumor burden.

In addition to DLBCL, PU-H71 has shown efficacy against lymphoma cells infected by Epstein–Barr virus (EBV) or Kaposi’s sarcoma associated herpes virus (KSHV) [114]. Specifically, viral and cellular proteins identified as Hsp90 clients were significantly downregulated, including many involved in NF-kB signaling (particularly the oncogenic vFLIP-ikkγ signaling complex that promotes KSHV carcinogenesis [115,116]), apoptosis, and autophagy. These in vitro effects were validated by potent in vivo activity against BC3 primary effusion lymphoma, a cancer cell line in which KSHV is the etiologic agent. Since many apoptosis pathway proteins in BC3 cells bound to Hsp90, PU-H71 was used in combination with the pan-BCL2 inhibitor obatoclax to assess their potential in vitro synergy. The agents subsequently demonstrated dramatic synergies, and may be indicative of a therapeutic combination that warrants further preclinical investigation.

5.5. Multiple myeloma

Multiple myeloma is a cancer of the antibody producing plasma cells in which collections of abnormal plasma cells accumulate in the bone marrow, interfering with hematopoiesis. Novel agents such as proteasome inhibitors (bortezomib and carfilzomib [117]) and immunomodulatory/antiangiogenic drugs (thalidomide, lenalidomide, and pomalidomide [118]) have increased progression free and overall long term survival, but the malignancy is still designated as incurable and the five year survival rate remains at ~45% [119]. As indicated by the efficacy of PU-H71 against multiple myeloma cells [120], Hsp90 is an attractive target for further preclinical evaluation. Several human myeloma cell lines including those that are resistant to corticosteroids and bortezomib are highly sensitive to PU-H71 [120]. The Hsp90 inhibitor activated the unfolded protein response by initiating a blockade of gp96 (the Hsp90 paralog found in the endoplasmic reticulum), as well as induced cell cycle arrest at the G1-S checkpoint and potentiated caspase-dependent apoptosis. Interestingly, a stable gp96 knockdown human myeloma cell line was found to be more resistant to PU-H71 and other Hsp90 inhibitors (17-AAG and 17-DMAG) even though these cells were more sensitive to conventional chemotherapy employed against multiple myeloma. PU-H71 also demonstrated notable synergistic interactions with bortezomib, dexamethasone, melphalan, and thalidomide, suggesting that the Hsp90 inhibitor could be used to supplement current anti-myeloma treatment protocols.

5.6. Ewing’s sarcoma

As shown in vitro against multiple myeloma, notable synergism may exist between PU-H71 and bortezomib. Being a potent inhibitor of the 26S proteasome, bortezomib prevents proteasome-mediated degradation of ubiquitinated proteins that may be vital for promoting apoptosis in malignant cells, and also assists in their activation. Supporting this notion are data that indicate the proteasome inhibitor induces G2/M cell cycle arrest and apoptosis by causing pro-apoptotic Bcl-2 phosphorylation and cleavage [121]. Interestingly, one of the most notable mechanisms of PU-H71 is indirect degradation of Hsp90 oncogenic proteins via the ubiquitin/proteasome system [39]. Although these two mechanisms of
action may at first appear to be conflicting, the synergy between PU-H71 and bortezomib is apparent, and has been reproduced in vitro and in vivo against Ewing's sarcoma [122], a predominately pediatric bone cancer characterized by small, round, and blue cells that are associated with highly undifferentiated malignancies [123].

PU-H71 potentiates a significant therapeutic window when its effects against multiple Ewing's sarcoma cell lines (A673, CADO-ES-1, RD-ES, SK-ES-1, SK-PN-DW, and TC71) were compared to normal and/or benign cells (HS-5 human bone marrow mesenchymal stromal cells, sarcoma derived benign mesenchymal stem cells, and human brain vascular pericytes) [124], again confirming the preferential activity of the Hsp90 inhibitor. As described in other cancer types [83, 124–126], PU-H71 also potentiated G2/M arrest, and depleted Hsp90 client proteins Akt, pERK, RAF-1, c-MYC, c-KIT, IGF1R, hTERT (human telomerase reverse transcriptase) and EWS-FLI1 (Ewing's sarcoma-Friend leukemia integration 1 transcription factor). In regards to potential drug synergy with bortezomib, FaCI (fraction affected–combination index) plots and isobolographic analyses confirmed synergism between PU-H71 and bortezomib. More importantly, Ewing's sarcoma xenograft–bearing mice fared much better when treated with the combination compared to the vehicle or either agent alone, providing a compelling rationale for further preclinical and the eventual clinical evaluation of PU-H71/bortezomib treatment protocols. The noted synergism between PU-H71 and bortezomib was attributed to the accumulation of ubiquitinated proteins that is potentiated by both agents, ultimately resulting in highly cytotoxic proteotoxic stress in affected cancer cells.

5.7. Hepatocellular carcinoma

Chemotherapeutic intervention against hepatocellular carcinoma has been historically difficult, as many traditional broad ranging cytotoxic agents are ineffective in patients afflicted with these malignancies [127]. Recent advances in molecular-based chemotherapy have yielded slight improvements in prognosis, as the versatile TKI sorafenib has shown efficacy in select individuals [128, 129]. Nevertheless, unresectable hepatocellular carcinoma typically portends a survival of less than 6 months post-diagnosis, although some patients fare much better [129]. Hsp90 inhibitors have not been extensively examined for their antitumor activity against this malignancy because geldanamycin-based analogs are known to induce dose-limiting liver toxicity (refer to section Initial Attempts to Target Heat Shock Protein 90 Using Semisynthetic Derivatives of Natural Products), which would further damage an organ already partially compromised from malignant progression. However, PU-H71 does not contain the benzoquinone moiety that has been linked to liver toxicity, thereby suggesting that the agent may yield some therapeutic benefit.

In the carcinogenesis of hepatocellular carcinoma, Hsp90 is vital for maintaining the malignant phenotype, as the protein promotes the aberrant expression and activity of crucial hepatocarcinogenesis–driving factors (IGF1R, HGF; hepatocyte growth factor receptor, Akt, CRaf, and CDK4) [124, 130]. PU-H71 was shown to potently inhibit the expression of these proteins, and caused G2/M arrest, as well as apoptosis [124]. Normal hepatocytes were exposed to these inhibitory concentrations, with little apparent toxicity observed. Perhaps most important was the fact that PU-H71 was retained in xenograft tumors at pharmacologically relevant concentrations, while being rapidly cleared from non-tumorous liver. Consequently, PU-H71 showed potent and prolonged in vivo Hsp90 inhibitory activity and reduced tumor growth without causing the liver toxicity that is associated with geldanamycin-based Hsp90 inhibitors. These data suggest that hepatocellular carcinomas may be responsive to PU-H71-mediated Hsp90 inhibition, warranting further preclinical investigation.

5.8. Lung carcinoma

Despite public awareness campaigns and novel molecular targeting therapies, lung carcinoma remains the most common cause of cancer-related death in men and women in the world, with approximately 14 million new cases and 8.2 million cancer related deaths reported in 2012 [131]. A particularly aggressive subtype of lung carcinoma is small cell lung carcinoma (SCLC), a poorly differentiated neuroendocrine tumor that is marked by the same small-blue-round-cell tumors found in Ewing’s sarcoma [132]. Unlike other forms of lung carcinoma, there has yet to be a clearly defined molecular target that responds well to currently approved mABs or TKIs. Although SCLC is notably sensitive to traditional cytotoxic chemotherapy, the malignancy quickly develops resistance, significantly limiting the treatment options available to patients [132]. Therefore, characterizing molecular oncogenic targets in SCLC that are particularly susceptible to drug inhibition would be of monumental importance in the clinical management of this malignancy.

Lo and behold, SCLC may be vulnerable to Hsp90 inhibition, as demonstrated by PU-H71 [133]. Unlike other cancers, SCLC does not have a functional death receptor pathway [134, 135], suggesting that apoptosis is predominately mediated through the intrinsic, mitochondrial associated pathway. Apoptotic signaling in mitochondria leads to membrane depolarization, resulting in activation of the effector caspase-3, poly(ADP-ribose) polymerase (PARP) cleavage, and DNA fragmentation. PU-H71, as well as other purine-based inhibitors considerably upregulated caspase-3 and caspase-7 activity and potentiated the dissolution of mitochondrial membrane potential [133], collectively indicating that Hsp90 inhibition can induce mitochondrial-mediated apoptosis in SCLC. Using the purine-based inhibitors as molecular probes, it was later uncovered that pharmacological inhibition of Hsp90s in SCLC cells frees Apaf-1 (apoptotic protease activating factor 1) to form an Apaf-1–caspase–9 complex [133]. This complex elicits apoptotic signaling after cytochrome c is released, which is triggered by Akt inactivation and subsequent BAD (Bcl-2–associated death promoter) dephosphorylation. Therefore, it appears that Hsp90 regulates apoptosis in SCLC by acting as a negative regulator of Apaf-1 and by controlling the P38K-Akt survival pathway.

These effects on apoptotic signaling appear to potentiate cell death in both drug sensitive and multidrug resistant SCLC cells in vitro (including H69AR, SKI-Ac3 and WBA), as PU-H71 and its congeners PU-H58 inhibited Hsp90 and caused substantial death in all tested cell lines [133]. The marked antineoplastic activity against SKI-Ac3 is particularly noteworthy, as the primary SCLC cell line was harvested from the malignant pleural effusion of a patient who had recurrent disease after being treated with cisplatin, etoposide, topotecan, gemcitabine, and whole-brain radiotherapy and had extensive metastases in the lungs, liver, lymph nodes, subcutaneous tissue, left adrenal gland, and brain. This prominent in vitro activity of PU-H71 was matched by its in vivo effects; in NC1-N417 xenografts, Akt inactivation and cleavage of PARP was detected as early as 6 h after PU-H71 administration, and the effects were still evident at 36 h. These effects occurred at pharmacologically relevant doses of PU-H71 that were retained in tumors, and significant tumor growth inhibition was noted at the time of termination. The study also likely documented the first instance of apoptosis in vivo mediated by a Hsp90 inhibitor, indicative of the potential of PU-H71 and other agents of this class may have against SCLC. It should be cautioned that since several currently approved chemotherapeutic agents can elicit initial dramatic responses in SCLC, much more extensive preclinical in vivo investigation is needed to
confirm the notable antitumor activity PU-H71 demonstrated in the study.

5.9. Thymic epithelial tumors

Thymic epithelial tumors constitute a diverse array of cancers originating from the thymus, the site of T-lymphocyte maturation. As such, malignancies of the thymus can be classified into two main groups; thymomas that contain immature non-malignant lymphocytes and the epithelial component retains histologic features specific to the normal thymus, and thymic carcinomas that exhibit a set of histologic features that are similar to those found in carcinomas of other organs, rather than a normal thymus [136–138]. These malignancies are uncommon and have a variable prognosis, as indicated by a comprehensive study of 1320 Japanese patients in which the five year survival rate was 67% for patients treated with total resection, 30% for patients treated with subtotal resection, and 24% for patients whose disease was inoperable [136]. Being a fairly rare cancer, the underlying molecular mechanisms of thymic epithelial tumors have been inadequately described, resulting in a dearth of available efficacious targeted therapies. Nevertheless, a recent study has suggested Hsp90 inhibition via the administration of PU-H71 may potentiate novel molecular-based therapeutic strategies in these malignancies [125].

As is the case with more prevalent cancers, thymic epithelial tumors of both the thymoma and thymic carcinoma classification rely on oncoproteins that are clients of Hsp90 [125]. Hsp90 inhibition significantly reduced the viability of thymoma (T1682) and thymic carcinoma (TC1889) cells, induced cell cycle arrest and apoptosis, and blocked invasiveness. PU-H71-mediated Hsp90 inhibition triggered the degradation of multiple oncogenic clients, including IGF1R, CDK4, and the inactivation of PI3K/Akt and RAF/Erk signaling. In addition, the use of PU-H71 as a molecular probe revealed that the IGF/IGF1R signaling axis contributed to the establishment of the anti-apoptotic phenotype of thymic epithelial tumors, with IGF1R being overexpressed in advanced malignancies derived from patient tumor samples. These data ultimately suggest that Hsp90 inhibition may be a viable therapeutic strategy in this uncommon neoplasm, but the assertion will need to be validated by in vivo examination.

6. Potential combination with Hsp70 inhibitors

As has been demonstrated in this review, Hsp90 is a viable antineoplastic target that can be effectively inhibited by PU-H71, as well as many other compounds. As a compensatory mechanism, many cancer cell lines increase expression of Hsp70, another molecular chaperone that is responsible for an assortment of physiological functions, including the regulation of apoptotic machinery (apoptosome, the caspase activation complex, and AIF; apoptosis-inducing factor) and have a similar role as Hsp90 in the proteasome-mediated degradation of apoptosis-regulating proteins. Further, Hsp70 is involved in carcinogenesis, as suggested by their constituency in the Hsp90 super-chaperone machinery, being associated with the Hsp90:HOP:Hsp70 complex responsible for stabilizing numerous oncoproteins [139–142]. Although inhibition of Hsp90 can potentiate potent antitumor activity, the effects can potentially be circumvented by the activity of HSF-1 (heat shock factor-1), the master regulator of the heat shock response, and the subsequent effects of Hsp70. HSF-1 is another Hsp90 client, but unlike oncoproteins, it becomes activated when Hsp90 is inhibited [143]. Activation of HSF-1 results in a feedback increase in Hsp70 levels, and due to the anti-apoptotic functionality of Hsp70 that inhibits both intrinsic and extrinsic apoptotic pathways, the feedback response limits the potency of Hsp90 inhibitors. Indeed, the anti-apoptotic function of Hsp70 is not limited to Hsp90 inhibition, and Hsp70 protects cells from many other apoptotic and necrotic stimuli [142,143]. Therefore, the concomitant inhibition of Hsp90 and Hsp70 could be particularly cytotoxic for malignant cells dependent on oncogenic addiction.

Unfortunately, it has been much more difficult to develop effective Hsp70 inhibitors. As with Hsp90, the protein contains an N-terminal ATPase domain, as well as a C-terminal client protein-binding domain that creates a ligand activated, bidirectional molecular switch. However, Hsp70 has a notably strong affinity for ADP and due to the high intracellular concentrations of ATP that are typically present [144,145], designing an agent that would be capable of competitive inhibition under these circumstances is particularly challenging. In addition, the binding mode of ATP to Hsp70 is such that important polar interactions are made with its β- and γ-phosphate groups that lie buried within a polar cavity of the protein [145]. Attempts to mimic these interactions have resulted in highly polar, but unwieldy molecules that have potent affinity for the ATP pocket, but possess minimal cellular activity [146].

Since inhibition of the ATPase activity in Hsp70 does not appear to be a viable therapeutic prospect as it is with Hsp90, potentially novel drug responsive targets need to be examined. A particularly interesting approach has been taken by the Chiosis laboratory, the same group that developed the first completely synthetic Hsp90 inhibitor. Since insufficient crystalline information had been acquired for human Hsp70 in an open, active conformation, Chiosis et al. used homology modeling to characterize a theoretical full-length human Hsp70 that captured the conformational flexibility of the protein [144]. These conformational analyses revealed another pocket, located near the N-terminus that was not evident nor entirely predicted by the available crystal structures of Hsp70. Chiosis et al. subsequently synthesized YK5 (N-(6-amino-2-(4,6-dimethoxy-2-(4-methylpiperazin-1-yl)-pyrimidin-5-ylthio)pyrimidin-4-yl)acrylamide), a novel 2,5-thiodipyrimidine and 5-(phenylthio)-pyrimidine compound that bound to the allostERIC site, and subsequently perturbed cancer viability through a Hsp70-mediated mechanism (Fig. 4A) [144]. Using YK5 as a model, other agents with closely related structures were developed to optimize Hsp70 inhibition, and have shown notable antineoplastic potential against multiple cancer cell lines (Kasumi-1, MDAMB-468, Mia-PaCa-2, MOLM-13, and SKBr3) in vitro [147,148]. These congeners contain either an acrylamide moiety that reacts with a cysteine residue in the allostERIC pocket once the molecule inserts into the binding site, or are functionalized at key positions along the carbon skeleton to elicit non-covalent interactions that together potentiate a binding affinity that is comparable to the inhibitory potential of the acrylamide-based agents (Fig. 4B). If the notable in vitro activity of these agents can be reproduced in vivo, potential therapeutic approaches involving the concomitant inhibition of Hsp90 and Hsp70 as a strategy by which to limit Hsp90 inhibitor resistance would be a sensible prospect warranting preclinical investigation.

7. Other potential concomitant therapeutic approaches

Already, PU-H71 has demonstrated notable synergistic activity with bortezomib in preclinical models of multiple myeloma and Ewing’s sarcoma [120,122]. There is an ever expanding field of proteasome inhibitors currently available to investigate other potential synergistic combinations, including carfilzomib, a semisynthetic analog of the natural product epoxomicin (isolated from Actinomycetales bacteria [149]) that irreversibly binds the 20S proteasome, potentiating a cytotoxic increase in polyubiquitin-terminated proteins associated with cell cycle arrest, apoptosis, and
Fig. 4. Interaction of Hsp90:HOP:Hsp70 complex with oncogenic proteins, and its inhibition using small molecule inhibitors. (A) Oncogenic proteins are processed through an intermediate complex containing Hsp90, Hsp70, and HOP, potentiating the conformational maturation of the oncoprotein, thereby promoting cell proliferation and survival. YK5 (molecular structure shown) is a 2,5'-thiodipyrimidine that interferes with the formation of a competent chaperone/onco-client complex, resulting in oncoprotein destabilization. Subsequent proteasome degradation of the oncoprotein elicits growth arrest, and potentially cell death via apoptosis. (B) Optimizing the inhibitory activity of 2,5'-thiodipyrimidines. Irreversible Hsp70 inhibitors of this drug class have an electrophilic acrylamide moiety that reacts with Cys267 of Hsp70 once the molecule conforms to a pocket located at the N terminal domain. Reversible inhibitors increase the already notable enthalpy of the appropriate fit by modifying key functional groups along the carbon skeleton to increase non-covalent interactions. This includes replacing the electrophilic acrylamide moiety with a functional group capable of forming an ionic bridge and filling the hydrophobic pocket occupied by an ethoxy group in 1 with a benzyloxy group. These modifications result in ligands with a reversible mode of binding that have comparable potencies to irreversible inhibitors. 1: N-(6-amino-2-(4,6-diethoxy-2-(4-methyl)piperazin-1-yl)-pyrimidin-5yl)thio)pyrimdin-4-yl)acrylamide. 2: 2-amino-N-(3-((4-(benzyloxy)-2-(4-methyl)piperazin-1-yl)-pyrimidin-5-yl)thio)phenyl)acetamide. Important functional moieties are highlighted with color coded outlines; blue (acrylamide moiety) and red (benzyloxy moiety).

Source: Data in panel B were acquired from [147]. (For interpretation of the references to color in this legend, the reader is referred to the web version of the article.)

the inhibition of neoplastic growth [150]. Carfilzomib was FDA approved in 2012 for patients with multiple myeloma that is refractory or has developed resistance to at least two prior therapies, including treatment with bortezomib and an immunomodulatory agent. Therefore, concomitant administration of PU-H71 and carfilzomib in addition to other chemotherapeutic agents may be a novel approach to generate longer, more durable responses in this currently incurable malignancy. Supporting this notion is the observation that PU-H71 also demonstrates synergism in vitro with dexamethasone, melphalan, and thalidomide, other agents currently used as standard therapy for multiple myeloma [120].
HER2/Neu overexpressing breast carcinomas and gastric adenocarcinomas also appear to be malignancies potentially susceptible to PU-H71-mediated drug synergism, as the oncoprotein is a faithful client of Hsp90. Indeed, other Hsp90 inhibitors have been tested in this regard with trastuzumab, with positive responses being observed. These studies should be extended to other agents indicated for HER2 malignancies, including pertuzumab (a mAb that binds a different epitope than trastuzumab), the antibody–drug conjugate (ADC) trastuzumab emtansine, and lapatinib, in addition to other potential TKIs. The concept of using PU–H71 and other Hsp90 inhibitors in combination with agents directed against specific oncoproteins that are Hsp90 clients is indeed an attractive prospect, and could be investigated in many different cancers. Agents of potential interest include, but are not limited to inhibitors of the following proteins; ALK, BCR-ABL, BRAF, HER2, JAK2, KIT, and mTOR.

In addition to its efficacy against HER2 positive breast carcinoma, PU-H71 has shown notable activity against TNBC [83]. Breast carcinomas of this subtype are typically treated with standard cytotoxic chemotherapy, including microtubule-directed mitotic inhibitors [151]. The fact that PU–H71 markedly increases the proportion of cells at G2/M, suggests that it could be combined with mitotic inhibitors such as vinca alkaloids, taxanes, epothilones, and eribulin to increase their antitumor activity. Clinical studies investigating the potential of Hsp90 and mitotic inhibitors have been previously described [39,63–65], and such studies involving PU–H71 are warranted.

Finally, a potentially novel concomitant chemotherapeutic strategy that could be explored at the preclinical level would be the combination of PU–H71 with microfilament-directed agents. Although there are no microfilament-directed agents currently approved by the FDA or other pharmaceutical regulating bodies, these compounds have intriguing therapeutic potential. Antineoplastic mechanisms of these agents include the potentiation of multinucleated cancer cells that are exquisitely sensitive to other forms of chemotherapy via inhibition of cytokinesis, reducing metastatic potential by limiting cell motility and adhesion (as well as inhibiting the secretion of glucosaminidases), and binding ATP-binding cassette (ABC) transporters, thereby enhancing the activity of other chemotherapeutic agents [1,152,153]. Due to their unique mechanisms of action, microfilament-directed agents have been shown to sensitize malignant cells to mTOR inhibitors, nucleic acid-directed agents and microtubule-directed agents [154–158]. These are some of the very agents that PU–H71 may synergize with, indicating that drug synergy may exist between Hsp90 inhibitors and microfilament-directed agents. This is supported by the G2/M cell cycle arrest activity observed after Hsp90 inhibition, as concomitant inhibition of mitosis at this critical checkpoint and at cytokinesis would likely reduce the ability of malignant cells to carry out a successful mitotic event. Several microfilament-directed agents of particular interest include cytochalasins and closely related chaetoglobosins, latrunculins, and jasplakinolide, each of which has been comprehensively described in prior reviews [1,152,159]. Although the synergism between Hsp90 inhibitors and microfilament-directed agents is purely theoretical at this point, preclinical investigation of this concomitant approach could validate potentially novel therapeutic strategies.

8. Conclusion

In an era of precision medicine that has enabled clinicians to select optimal therapies based on the genetic profile or other molecular/cellular analyses of the patient’s tumor, the chaperone provides a novel target by which to preferentially damage neoplastic cells. Proteins that constitute this unique molecular family are vital to the carcinogenesis of many malignancies, and provide a method by which to inhibit specific oncoproteins without the use of mAbs or TKIs. The molecular chaperone that has shown the most therapeutic potential, Hsp90, can be successfully targeted through the use of compounds that potently inhibit its ATPase functionality. However, preliminary attempts to target Hsp90-dependent tumors in the clinical setting with derivatives of natural products have so far been unsuccessful due to their unfavorable pharmacokinetic properties and toxicity profiles. While second generation natural product analogs have shown potential, it may be necessary to develop completely synthetic small molecule inhibitors that do not rely on the key functional groups of geldanamycin or radicicil.

Purine-based inhibitors were the first class of fully synthetic anti-Hsp90 agents developed, and have been useful in probing the oncogenic function of Hsp90 due to their remarkable specificity for the protein. One congener in particular, PU–H71, has shown notable preclinical antineoplastic activity in a variety of cancer types, and is now being examined at the clinical level. Unlike some of its predecessors, PU–H71 has demonstrated marked specificity for malignant cells, displaying minimal toxicity to normal tissue. Further, the agent has the added benefit of having a replaceable iodide functional group, enabling the PET radionuclide 124I to be inserted for clinical pharmacological examination. Perhaps most importantly is the ability of PU–H71 to synergize with a variety of currently approved chemotherapeutic agents, suggesting the Hsp90 inhibitor could be used to supplement current treatment protocols.

Nevertheless, PU–H71 is only in the initial stages of clinical examination and its potential as a pharmaceutical drug remains uncertain. Targeting Hsp90 and other molecular chaperones is a fairly novel therapeutic strategy, and it will likely be years before an inhibitor of this class is clinically validated. Potential dual inhibition of the Hsp90:Hop:Hsp70 complex via Hsp90 and Hsp70 inhibitors is a potentially exciting prospect, but will need to be thoroughly examined in preclinical models before it can meet clinical scrutiny. The potential payoff is immense, as it has been elucidated that the expression of molecular chaperones is inherently different in normal and malignant cells, providing a rational approach to indirectly target oncoproteins and aberrant signaling cascades. Critical assessment of these novel antineoplastic agents will hopefully potentiate a new avenue of cancer therapy, and increase the rate at which sustainable remissions are seen in a select group of malignancies.

Conflicts of interest

The author declares no conflicts of interest.

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