



Review

Advances in the clinical development of heat shock protein 90 (Hsp90) inhibitors in cancers[☆]

Komal Jhaveri^a, Tony Taldone^b, Shanu Modi^a, Gabriela Chiosis^{a,b,*}

^a Department of Medicine, Breast Cancer Service, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

^b Department of Molecular Pharmacology and Chemistry, Sloan-Kettering Institute, New York, NY, USA

ARTICLE INFO

Article history:

Received 18 August 2011

Received in revised form 21 October 2011

Accepted 23 October 2011

Available online 29 October 2011

Keywords:

Heat shock protein 90

Chaperone

Cancer

Targeted therapy

ABSTRACT

Hsp90 is an ATP dependent molecular chaperone protein which integrates multiple oncogenic pathways. As such, Hsp90 inhibition is a promising anti-cancer strategy. Several inhibitors that act on Hsp90 by binding to its N-terminal ATP pocket have entered clinical evaluation. Robust pre-clinical data suggested anti-tumor activity in multiple cancer types. Clinically, encouraging results have been demonstrated in melanoma, acute myeloid leukemia, castrate refractory prostate cancer, non-small cell lung carcinoma and multiple myeloma. In breast cancer, proof-of-concept was demonstrated by first generation Hsp90 inhibitors in combination with trastuzumab mainly in human epidermal growth factor receptor 2 (HER2) + metastatic breast cancer. There are a multitude of second generation Hsp90 inhibitors currently under investigation. To date, however, there is no FDA approved Hsp90 inhibitor nor standardized assay to ascertain Hsp90 inhibition. This review summarizes the current status of both first and second generation Hsp90 inhibitors based on their chemical classification and stage of clinical development. It also discusses the pharmacodynamic assays currently implemented in clinic as well as other novel strategies aimed at enhancing the effectiveness of Hsp90 inhibitors. Ultimately, these efforts will aid in maximizing the full potential of this class of agents. This article is part of a Special Issue entitled: Heat Shock Protein 90 (HSP90).

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1. Introduction

Hsp90 is a chaperone that functions in the correct folding of its client proteins to their active conformation. Hsp90 is an ATPase and accomplishes its role through a complex cycle regulated by the binding and hydrolysis of ATP as well as by numerous co-chaperones (Hsp70, HOP, Cdc37, p23, Aha1). Inhibition of the Hsp90 chaperone cycle causes client proteins to undergo ubiquitination and subsequent degradation by the proteasome [1,2]. Because many of its clients include oncoproteins with important functions in the development and promotion of cancer, Hsp90 is an important target in cancer therapy [3].

The discovery of Hsp90 as the target of anticancer activity of geldanamycin (GM) (Fig. 1) sparked much interest in the inhibition of Hsp90 as a strategy for the treatment of cancer. This interest has resulted in intense efforts from both industry and academic centers to develop clinically viable small molecule Hsp90 inhibitors [4]. As a result of these efforts, there are numerous small-molecule Hsp90 inhibitors that are currently undergoing clinical investigation in a wide range of malignancies. Of the numerous ways of potentially

inhibiting the chaperone cycle, it should be noted that each of the molecules currently in clinical trials inhibits the ATPase activity of Hsp90 by binding to the N-terminal domain nucleotide binding pocket [5,6].

The clinical development of Hsp90 inhibitors, from GM to the newest agents, has been and will continue to be driven by the optimization of pharmaceutical properties including pharmacokinetic, pharmacodynamic and toxicological. Even though there are currently no approved Hsp90 targeted agents, research during this past decade has been very promising as there has been considerable progress on many fronts including improved formulations and the introduction of chemically distinct Hsp90 inhibitors with improved properties. These advances together with the increased biological knowledge of Hsp90 can help to fulfill the promise of this target and potentially result in the approval of the first-in-class agent.

2. Clinical studies with Hsp90 inhibitors in cancers

While the molecules currently advanced to clinical trials constitute a diverse array of structures, a close inspection of them shows that they can in general be classified according to their similarity to GM, radicicol (RD) or to the purine-scaffold. Only SNX-5422 falls outside any of these designations (Fig. 1 and Table 1). As is often the case in drug discovery, natural products play a prominent role in the discovery of lead agents. In the case of Hsp90, GM, RD and

[☆] This article is part of a Special Issue entitled: Heat Shock Protein 90 (HSP90).

* Corresponding author at: Department of Molecular Pharmacology and Chemistry, Sloan-Kettering Institute, USA. Tel.: +1 646 888 2235.

E-mail address: chiosisg@mskcc.org (G. Chiosis).

ATP have each had a profound effect on the development of small-molecule Hsp90 inhibitors. While none of these are suitable as a clinical agent, they have each served as good lead molecules or starting points for most of the agents in clinical trials.

2.1. Geldanamycin derivatives

GM is a benzoquinone ansamycin first isolated from a fermentation broth of *Streptomyces hygroscopicus* in 1970 (Fig. 1) [7]. Originally pursued as an antibiotic, its anticancer properties were discovered following a phenotypic screening of compounds capable of reversing v-src oncogene transformed cells [8]. Initially, it was believed to be a direct inhibitor of src kinase, however, it was later shown to directly bind to Hsp90 and interfere with Hsp90-v-src heterocomplex formation [9]. Further work showed that GM inhibits the ATPase activity of Hsp90 by competing with ATP for binding to the N-terminal domain nucleotide binding pocket [10,11], resulting in ubiquitin mediated proteasomal degradation of its client proteins [1,2,12].

Despite its potent anti-tumor effects, GM was never evaluated in clinical trials because of its poor “drug-like” properties including poor solubility, limited in vivo stability and significant hepatotoxicity in animals [13,14]. Structural features of GM include a quinone ring (depicted in blue, Fig. 1), moiety contributing to the observed hepatotoxicity, along with a pendant macrocycle containing a carbamate group essential for binding (Fig. 1). Additionally, GM contains a non-essential methoxy group on C-17 of the quinone ring (depicted in red, Fig. 1) that can readily be substituted with amines. This approach was used in an attempt to overcome the liabilities associated with GM and resulted in the preparation of many analogs including those that have entered clinical trials (Table 1, entry 1–4).

2.1.1. 17-AAG (17-allyl-17-demethoxygeldanamycin)

Substitution in GM of the methoxy group with an allylamino group resulted in 17-AAG (Table 1, entry 1), a compound with biological activity similar to GM but with an improved toxicity profile [15]. In fact, 17-AAG was the first Hsp90 inhibitor selected by the Cancer

Therapy Evaluation Program (CTEP) of the National Cancer Institute (NCI) for clinical studies in cancer and provided proof-of-concept for Hsp90 inhibition in humans. In these studies, 17-AAG has been evaluated using weekly, weekly for 3 weeks (28 day cycle), daily×5 (21 day cycle), and daily×3 (14 day cycle), twice weekly (day 1, 4), and on days 1, 4, 8, 11 (21 day cycle) schedules [16–23]. Toxicity of 17-AAG in these trials was dose and schedule dependent, with hepatotoxicity being more prominent with daily administration. Other toxicities were primarily diarrhea and fatigue. Because of its poor solubility and in order to administer 17-AAG intravenously, the NCI had developed a dimethyl sulfoxide and egg phospholipid vehicle, however, at doses greater than 100 mg/m², the toxicities due to this vehicle (bad odor, nausea, anorexia) were as significant as the toxicities from the drug itself. Pharmacokinetic (PK) studies that were incorporated in these phase I trials suggested that serum concentrations greater than those required for depletion of Hsp90 client proteins in vitro and in xenograft models could be achieved at well-tolerated doses and schedules.

Pharmacodynamic (PD) assays in these trials included peripheral blood mononuclear cell (PBMC) studies and limited tumor biopsies, as discussed further in Section 3 of this review [16,20]. Despite the evidence of at least partial target modulation in these phase I trials, there were no complete or partial responses (CR or PR respectively) by RECIST (Response Evaluation Criteria in Solid Tumors). Several patients with melanoma, breast cancer, prostate cancer and renal cell carcinoma did have stable disease (SD). Disappointingly, single agent phase II studies using 17-AAG once weekly (300–450 mg/m²) in prostate cancer or melanoma or twice weekly (220 mg/m²) in renal cell carcinoma patients also showed no objective responses with this dose and schedule of 17-AAG [24–26].

In patients heavily pretreated for relapsed/refractory multiple myeloma, single agent 17-AAG administered on days 1, 4, 8, and 11 every 3 weeks was well tolerated with one minimal response (MR), with a progression-free survival (PFS) of 3 months, a 41% decline in urine M protein, and a 33% decrease from baseline in serum M protein. Fifteen other patients had SD with a median PFS of 2.1 months [27].

Limited success of 17-AAG in these studies was attributed to poor solubility, poor patient enrichment for those most likely to benefit based on preclinical experience (for e.g. patients with HER2 amplified breast cancer), suboptimal target inhibition and off-target toxicities from 17-AAG and its DMSO formulation.

Improving the formulation and the delivery of 17-AAG in disease specific population has led to encouraging results. Kosan Biosciences developed a Cremophor containing formulation of 17-AAG (tanespimycin, KOS-953) as an injectable suspension. The first solid tumor trial with this formulation was a phase I/II study of 17-AAG and trastuzumab. The phase I portion was open to all tumor histologies. Of the 25 patients enrolled, 15 had HER2+ metastatic breast cancer. Patients were enrolled onto 4 dose cohorts (225–450 mg/m²) and treated with both agents on a weekly schedule. Dose limiting toxicity (DLT) included fatigue, nausea, anorexia and thrombocytopenia. With the weekly schedule, hepatotoxicity was minimal. Anti-tumor activity was seen only in patients with HER2+ metastatic breast cancer with 1 confirmed PR, 4 MR and 4 patients with SD for more than 4 months [28]. In order to obtain a more precise estimate of activity, the phase II trial enrolled 31 patients with HER2+ metastatic breast cancer who were previously refractory to trastuzumab therapy [29]. Patients were treated with 450 mg/m² of tanespimycin with trastuzumab weekly. The primary endpoint was response rate (RR) by RECIST criteria. Most common toxicities were diarrhea, fatigue, nausea and headache. The overall RR was 22% and the clinical benefit rate (CR+PR+SD) was 59% with a median PFS of 6 months (95% CI: 4–9) and median overall survival (OS) of 17 months (95% CI: 16–28). The effectiveness noted in this phase II trial could be due to potent target degradation (i.e. HER2+) due to the combination of these drugs and perhaps because Hsp90 inhibitors can overcome or delay the initial resistance to trastuzumab [30].

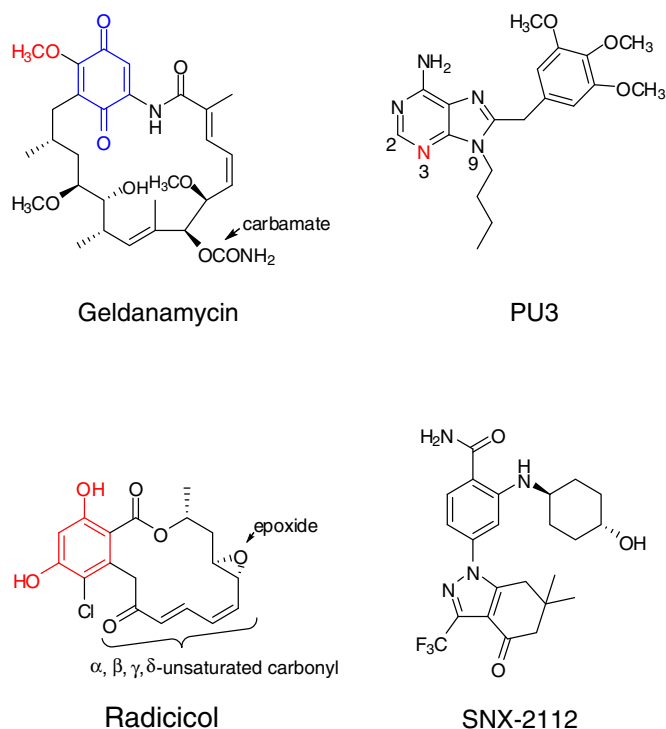


Fig. 1. Structures of representative Hsp90 inhibitor chemical classes.

Table 1
Hsp90 inhibitors in clinical evaluation.

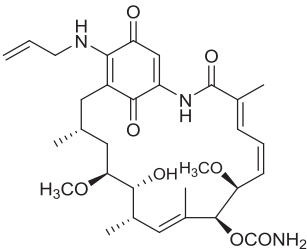
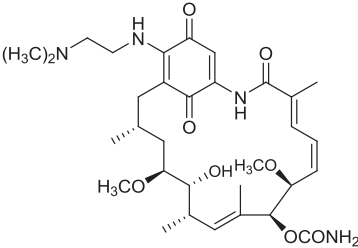
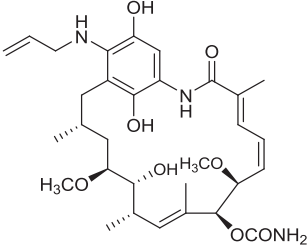
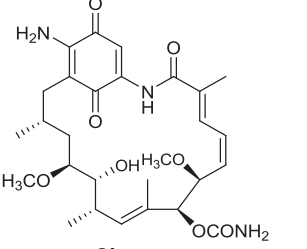
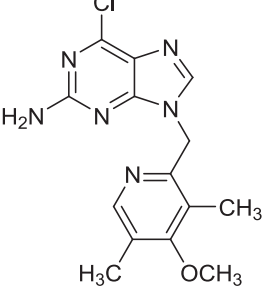
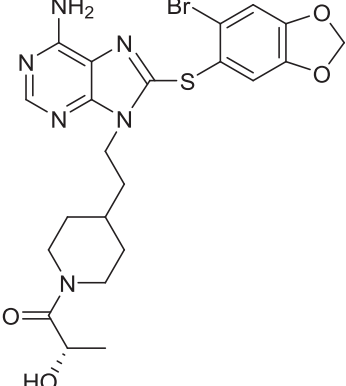
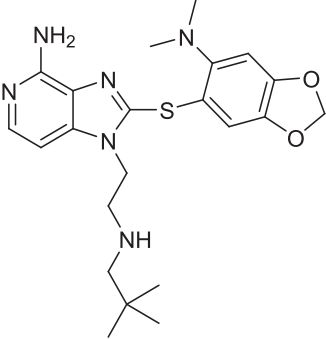
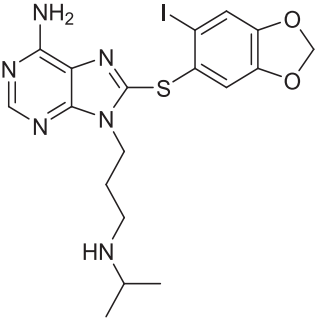
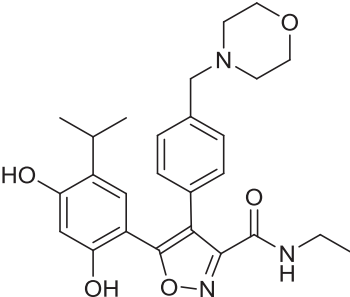
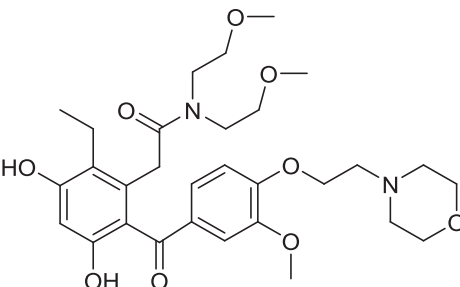
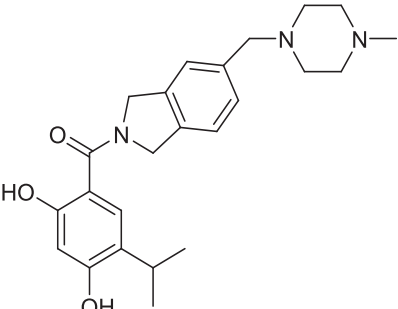
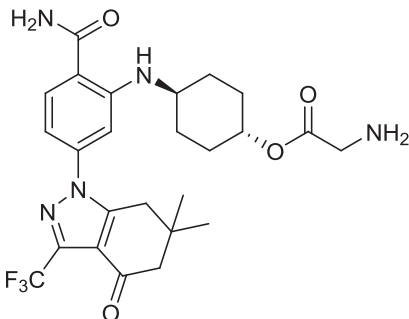
	Inhibitor	Company	Structure	Class	Route	Phase
1.	Tanespimycin (17-AAG, KOS-953)	Kosan Biosciences/ Bristol-Myers-Squibb		GM	IV	III
2.	Alvespimycin (17-DMAG)	Kosan Biosciences/ Bristol-Myers-Squibb		GM	IV Oral	I
3.	Retaspimycin (IPI-504)	Infinity Pharmaceuticals		GM	IV	III
4.	IPI-493	Infinity Pharmaceuticals		GM	Oral	I
5.	CNF2024/BIIIB021	Biogen Idec		Purine	Oral	II
6.	MPC-3100	Myriad Pharmaceuticals/ Myrexis		Purine	Oral	I

Table 1 (continued)

	Inhibitor	Company	Structure	Class	Route	Phase
7.	Debio 0932 (CUDC-305)	DebioPharm		Purine-like	Oral	I
8.	PU-H71	Samus Therapeutics		Purine	IV	I
9.	Ganetespib (STA-9090)	Synta Pharmaceuticals	Not reported	Resorcinol-Triazole	IV	II
10.	NVP-AUY922 (VER-52269)	Novartis		Resorcinol-Isoxazole	IV	II
11.	HSP990	Novartis	Not reported but claimed as a follow up compound to NVP-AUY922	Not reported	Oral	I
12.	KW-2478	Kyowa Hakko Kirin Pharma		Resorcinol	IV	I
13.	AT13387	Astex		Resorcinol	IV Oral	I

(continued on next page)

Table 1 (continued)

	Inhibitor	Company	Structure	Class	Route	Phase
14.	SNX-5422	Serenex/Pfizer		Indazol-4-one	Oral	I
15.	DS-2248	Daiichi Sankyo Inc	Not reported	Not reported	Oral	I
16.	XL888	Exelixis	Not reported	Not reported	Oral	I

Similar to these responses for tanespimycin in solid tumors, strong synergistic effect of anti-tumor activity was also noted with combination of tanespimycin and bortezomib in multiple myeloma. In a phase I/II study, 72 patients with relapsed and refractory multiple myeloma were treated with this combination (tanespimycin 100–340 mg/m² and bortezomib 0.7–1.3 mg/m²) [31]. The highest dose for tanespimycin tested in this study was 340 mg/m² and no DLT was identified. Importantly, there were no cases of grade 3 peripheral neuropathy. Anti-tumor activity was observed with overall RR≥MR in 48% of bortezomib-naïve, 22% of bortezomib-pretreated and 13% of bortezomib refractory patients; median duration of response for all responders was 12 months. The phase II study of this combination, although an extension of the phase I/II study, deployed a broader tanespimycin dosage range (50–340 mg/m²). Unlike the previously described phase I/II study, this phase II trial involved a slightly different population, in that they excluded bortezomib-naïve patients and patients with no measurable multiple myeloma [32]. Twenty-two patients were administered with this combination regimen on days 1, 4, 8, 11 in each 3-week cycle. Most common adverse events were fatigue, diarrhea, nausea, constipation and vomiting. Liver toxicity related adverse events were noted in 4 patients, which was manageable and reversible, and thrombocytopenia was the most common hematological adverse event. Peripheral neuropathy is a significant toxicity of single agent bortezomib therapy. However, there were low rates of peripheral neuropathy, in particular grade 3 peripheral neuropathy, seen with combination of tanespimycin and bortezomib. This favorable effect of both decreased rate and severity of peripheral neuropathy correlated well with the pre-clinical observation that tanespimycin has a neuroprotective effect against bortezomib-induced peripheral neuropathy [33]. Overall RR was 14% (2 PR and 1 MR) and an additional 10 patients had SD. This led to further development of tanespimycin in phase III trials for potential treatment of multiple myeloma. However, Kosan, acquired by Bristol-Myers-Squibb in 2008, suspended this phase III trial for non-clinical reasons [34].

Because Hsp90 can protect cells under stress conditions, Hsp90 inhibitors have the ability to sensitize cells to the toxic effects of chemotherapy (including taxanes, anthracyclines, nucleoside analogs and topoisomerase I inhibitors and lead to synergistic activity) and radiation therapy. One possible mechanism by which Hsp90 inhibitors are thought to enhance the cytotoxicity of chemotherapy is by depleting the checkpoint kinase1 (chk1). Chk1 is a Hsp90 client protein that regulates progression through the cell cycle and is particularly critical for the S and G2-M phases [35]. Hsp90 inhibitors degrade chk1 which in turn abrogates G1/S arrest induced by gemcitabine [35] and G2-M checkpoint induced by topoisomerase I inhibitors such as irinotecan [36]. Nguyen et al. showed 5–22 fold enhancement of paclitaxel cytotoxicity when combined with Hsp90 inhibitors [37]. Breast cancer cell lines exposed to Hsp90 inhibitors

were sensitized to the apoptotic effects of paclitaxel by activation of caspase-3 and -9 [38]. Cells with intact retinoblastoma gene (RB) that was exposed to 17-AAG followed by paclitaxel underwent G1 arrest and enhanced apoptosis. This schedule dependence was not noted in cells harboring a mutated RB. Akt inactivation is another mechanism by which Hsp90 inhibitors sensitize tumor cells to induction of apoptosis by paclitaxel [39]. These studies formed the rationale for combination phase I trials of 17-AAG with paclitaxel [38,40], docetaxel [41], cisplatin [42], gemcitabine-cisplatin [43] and irinotecan [44].

Similarly, Hsp90 inhibitors are thought to enhance the radiosensitivity of many tumor cell lines. Raf-1, Akt and HER2 are radioresponse proteins that protect against radiation induced cell death. Hsp90 inhibition causes degradation of the radioresponse proteins, increases apoptosis and enhances G2 arrest [45]. Lastly, in an attempt to show synergistic activity by simultaneously inhibiting the Raf-signaling pathway, a phase I trial evaluated the combination of 17-AAG with Sorafenib and showed clinical activity in renal cell carcinoma and melanoma patients [46].

Alternatively, agents such as cisplatin may enhance the activity of Hsp90 inhibitors [47]. Under non-stress conditions, heat shock factor 1 (HSF1) binds with Hsp90, but when an Hsp90 inhibitor blocks the target, HSF1 is released, thereby causing a heat shock response that potentially limits the effect of Hsp90 inhibition. Cisplatin blocks the HSF1 mediated heat shock response by blocking HSF1 binding to the promoter region of the transcription factor [42,48].

Despite, these early encouraging results, the development of 17-AAG was halted in July 2008. However, more recently, 17-AAG was successfully converted to nanoparticle albumin-bound, nab-17AAG/ABI-1010 to improve the safety and efficacy over their surfactant and solvent based counterparts [49]. This agent is currently being evaluated in a phase I trial in combination with Abraxane in patients with advanced solid tumors. Patients will be administered ABI-1010 intravenously weekly for 3 weeks of a 28-day cycle; results of this trial are awaited [50].

2.1.2. 17-DMAG (17-desmethoxy-17-N,N-dimethylaminoethylaminogel danamycin)

Substitution of the C-17 methoxy group of GM with N, N-dimethylethylamine resulted in 17-DMAG (Table 1, entry 2). The presence of an ionizable amino group resulted in increased water solubility, better oral bioavailability and equal or greater anti-tumor activity compared to 17-AAG [51,52]. 17-DMAG was developed by the NCI and Kosan and entered phase I clinical trials in 2005. Various schedules were evaluated including twice weekly [53], daily×3 or 5 days every 3 weeks (1.5–46 mg/m²) [54], weekly (2.5–80 mg/m²) [55], twice weekly×2 weeks and every 3 weeks (8–32 mg/m²) [56]. Toxicity in these trials included peripheral neuropathy [53], renal dysfunction [53,55], fatigue [55], cardiotoxicity [56], ocular adverse

events comprised of blurred vision, keratitis, dry eyes [55], pneumonitis with dyspnea and thrombocytopenia [56]. Efficacy assessment included 1 CR (castrate refractory prostate cancer) and 1 PR (melanoma) [55] and 3 CR in acute myeloid leukemia [56]. There was also one phase I trial of orally administered 17-DMAG wherein patients were administered 17-DMAG either daily or every other day for 4 out of 6 weeks [57]. In the 28 patients treated, no DLT was observed and common drug-related toxicities included fatigue, anorexia, proteinuria and peripheral edema. Bioavailability in 14 patients was 51% and 49% on day 1 and 21 respectively and was not dose-dependent. Early signs of activity included SD in hemangioendothelioma, melanoma and renal cell carcinoma.

17-DMAG (60–100 mg/m²), administered weekly has also been investigated in combination with trastuzumab (phase I study) in 28 patients with metastatic breast cancer and metastatic ovarian cancer. DLT was grade 3 keratitis, which was reversible, and 1 patient had a decline in left ventricular ejection fraction. Anti-tumor activity was seen in patients with HER2+ metastatic breast cancer (1 PR, 1 MR and 5 patients with SD). Tumor regression including CR was also noted in 1 patient with HER2+ metastatic breast cancer and another patient with ovarian cancer (HER2 status unknown) [58].

Despite the CR with 17-DMAG in patients with acute myeloid leukemia, Kosan discontinued its development in 2008 to “commit resources to the development of 17-AAG for the treatment of breast cancer as a result of a comparative analysis with 17-AAG based on several factors, including clinical experience to date, strength of intellectual property protection and risk, and time to commercialization” [59].

2.1.3. IPI-504 [17-allylamino-17-demethoxygeldanamycin hydroquinone hydrochloride]

In a different approach, Infinity Pharmaceuticals developed IPI-504 (retaspimycin) (Table 1, entry 3), a new GM analog, which is the water-soluble hydroquinone hydrochloride salt derivative of 17-AAG [60]. IPI-504 can be prepared by reduction of 17-AAG with sodium dithionite followed by conversion to its hydrochloride salt. IPI-504 and 17-AAG actually exist in a redox equilibrium in vivo between the hydroquinone (as in IPI-504) and quinone (as in 17-AAG) forms through the action of oxidoreductases. The hydroquinone form in IPI-504 is a more potent inhibitor of Hsp90 and is likely the more relevant form in vivo. Additionally, since the quinone ring has been identified as a primary cause for the hepatotoxicity of GM, its reduction to a hydroquinone has predictably resulted in diminished toxicity. For a number of reasons, including improved PK and toxicity properties, IPI-504 appears to be the most promising inhibitor derived from the ansamycin class [61]. Various phase I–III trials have been conducted in patients with multiple myeloma, non-small cell lung cancer, castrate resistant prostate cancer, breast cancer and gastrointestinal stromal tumor.

In the phase I trial in patients with relapsed and refractory multiple myeloma, IPI-504 was infused on days 1, 4, 8, 11 of a 21-day cycle. The primary objective was MTD which was determined to be 400 mg/m² [62].

A phase I/II trial was conducted in 12 patients with non-small cell lung cancer after they had progressed on epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI). Patients were administered with IPI-504 twice weekly on a 4 week cycle at doses (150 to 300 mg/m²). DLT was increased liver enzymes and fatigue. Of the 9 patients who were evaluable, 7 had best response of SD. Of the 4 patients who had evaluable disease by ¹⁸F-fluorodeoxyglucose (FDG) positron emission tomography (PET), 2 had SD and 2 had PR [63]. This promising activity led to the phase II portion of the study which evaluated 76 patients at 400 mg/m² administered twice weekly on a 21 day cycle [64]. The primary objective was overall RR; secondary aims included safety, PFS and analysis by molecular subtypes. The most common side effects were fatigue, diarrhea and

nausea. Grade 3 or higher liver function abnormalities were observed in 9 patients. Overall RR was 7% (5 PR) with 10% in patients who were EGFR-wild type and 4% in those who had EGFR mutations. Although the target overall RR was 20% for the two EGFR groups, 2 of the 3 patients with oncogenic rearrangement of the anaplastic lymphoma kinase (ALK) gene also had PR and the third had SD for 7.2 months with 24% reduction in tumor size. This was the first trial that showed that Hsp90 inhibitors have clinical activity in non-small cell lung cancer, particularly among those with ALK rearrangements [64].

A phase Ib trial evaluated IPI-504 in combination with docetaxel in 16 patients with advanced solid tumors of which 6 were non-small cell lung cancer. Patients were treated with 300–550 mg/m² of IPI-504 in combination with 75 mg/m² of docetaxel on a once every 3 weeks schedule. DLT included fatigue, neutropenia, asymptomatic bradycardia, increase in liver enzymes with acute respiratory distress syndrome; all of which were reversible on trial. MTD was identified as 450 mg/m² [65]. In the expansion phase, 23 patients with non-small cell lung cancer were treated with 300 mg/m² of IPI-504 in combination with 75 mg/m² of docetaxel weekly in a 21-day cycle. Among the 23 patients, there was no drug-related hepatotoxicity. Gastrointestinal toxicity was low grade and easily manageable. The overall RR was 26% (6 PR, 7SD). Higher response rates were seen in heavy smokers and those with squamous cell histology. Based on these data, an adaptive randomized phase II study is underway comparing IPI-504 and docetaxel versus placebo+docetaxel in pretreated patients with non-small cell lung cancer and a smoking history. This trial is designed to identify and validate potential predictive biomarkers of the activity of combination treatment [66].

A single agent phase II trial evaluated the safety and efficacy of IPI-504 in two groups of men with castrate resistant prostate cancer. Group A was chemotherapy-naïve patients and group B included patients who had disease progression on previous docetaxel-based therapy. IPI-504 was administered at 400 mg/m² twice weekly on a 21-day cycle. Of the 19 patients enrolled in this study, 15 had prior docetaxel-based therapies. There was no prostate specific antigen (PSA) or RECIST responses although one patient remained on study for 9 cycles with 48% PSA decline. Serious adverse events were noted in group B which included grade 5 hepatic failure and grade 5 ketoacidosis and the study concluded that further evaluation in this population was not warranted [67].

Recently, the results of a phase II trial of IPI-504 in combination with trastuzumab in patients with metastatic HER2+ metastatic breast cancer were presented at the 2011 Annual ASCO scientific meeting. 26 patients were treated with 300 mg/m² of IPI-504 weekly in combination with trastuzumab 6 mg/kg once every 3 weeks. Endpoints of this study included overall RR and tolerability of IPI-504. The trial used a Simon two-stage design with prespecified criteria; if the DLT rate was <25% and at least 2 responses were seen, then the trial would be expanded. No DLTs were reported. The most common adverse events noted were fatigue, nausea and diarrhea. Among the 20 patients evaluable for efficacy, 1 PR was observed and 1 patient had SD for 6 months. Thus, there was modest clinical activity but the trial did not meet the prespecified criteria for trial expansion [68].

Patients with TKI-resistant gastrointestinal stromal tumor were treated with IPI-504 in a phase I dose-escalation trial. The initial trial design included only 45 patients with metastatic and unresectable gastrointestinal stromal tumor but later, 18 patients with soft tissue sarcomas were also included. IPI-504 was evaluated in five dose levels administered either twice weekly for 2 weeks followed by 1 week off or twice weekly for 3 weeks in continuous 21 day cycles. The objectives of this trial were to determine the MTD, find the dose and schedule for subsequent trials and to assess the biologic activity of IPI-504 with anatomic and FDG-PET imaging. FDG-PET imaging was done at baseline, day 11 and 21. IPI-504 was well tolerated up to 400 mg/m² twice weekly followed by 1 week off. DLT was headache and myalgia. Other common adverse events included fatigue,

diarrhea and elevated liver enzymes. Of the 36 patients evaluable for efficacy on this schedule, 67% had SD and 3% had a PR. For the 18 patients evaluable by FDG-PET, 22% had a PET PR and 66% had SD. Median PFS was 12 weeks [69].

The RING (retaspimycin in GIST) trial was an international phase III, double blinded, placebo controlled trial of IPI-504 in patients with refractory gastrointestinal stromal tumor that had failed previous therapies with either imatinib or sunitinib. There was no limit on previous therapies. Target enrollment was 200 patients, however the study was terminated after 46 patients were enrolled as a higher mortality rate was observed for patients in the treatment arm. Review of the patients' demographics revealed that the higher mortality rate was observed in those enrolled in the treatment arm who had received three or more therapies since their initial diagnosis and had more advanced disease compared to patients enrolled in the earlier IPI-504 trials [70]. Due to the safety concerns and the increased mortality rate in this population, and because 17-AAG and IPI-504 are interconvertible *in vivo*, the phase III trial of 17-AAG was also terminated [61].

2.1.4. IPI-493 (17-desmethoxy-17-amino geldanamycin)

IPI-493, the primary active, long-lived metabolite of 17-AAG was developed by Infinity Pharmaceuticals as an oral formulation (Table 1, entry 4). However, IPI-493 suffers from poor pharmaceutical properties including low solubility and is difficult to administer in pharmacologically relevant doses. Because drug exposure of IPI-504 was superior to IPI-493 Infinity has halted the development of this agent to focus exclusively on retaspimycin [71].

2.2. Purine and purine-like analogs

The rational design of Hsp90 inhibitors became possible following the availability of X-ray crystal structures of Hsp90 bound to ATP/ADP as well as to GM and RD. Hsp90 has a unique fold among most other ATPases resulting in a distinctive conformation adopted by bound nucleotide that has enabled for its selective inhibition by small-molecules over other ATP/ADP binding proteins [72]. Taking advantage of this unique fold and using a structure-based approach, Chiosis et al. designed the first reported synthetic Hsp90 inhibitor, PU3 (Fig. 1), based on the purine-scaffold by incorporating features that enabled the molecule to adopt a bent conformation [73]. As the prototype for the purine-scaffold class, PU3 has been optimized by numerous groups through a variety of strategies to result in potent and selective inhibitors with improved pharmaceutical properties. Those that have advanced to clinical trials include the purines CNF2024/BII021, MPC-3100, and PU-H71 as well as the purine-like Debio 0932 (CUDC-305) (Table 1, entry 5–8). Each of these molecules has in common an $-NH_2$ group attached to a purine or purine-like core and an aryl moiety separated by approximately 5 Å as critical elements for binding to Hsp90. The aryl moiety is attached to the purine or purine-like core by a one atom linker [74].

2.2.1. CNF 2024/BII021

CNF 2024/BII021 was discovered and first developed by Conforma Therapeutics and later by Biogen Idec (Table 1, entry 5) [75–77]. CNF2024/BII021 is unique amongst the other members in this class in that the aryl moiety is attached to the 9-position of the purine. In order to maintain the critical distance of 5 Å, the amino group was moved to the 2-position.

This agent has been evaluated in phases I and II clinical trials. A phase I clinical trial evaluated BII021 administered orally daily for 3 weeks in a 28-day cycle in patients with chronic lymphocytic leukemia versus orally twice weekly for 3 weeks in a 28-day cycle in patients with advanced solid tumors. MTD was determined at 800 mg twice weekly and DLT was syncope and dizziness in patients with advanced solid tumors. Other grade 3 or 4 toxicities included

fatigue, hyponatremia and hypoglycemia. There was one incidence of grade 3 abnormal liver enzymes in a patient with chronic lymphocytic leukemia. Efficacy assessment revealed that one patient with chronic lymphocytic leukemia had a 39% reduction in the lymph node size at 25 mg dose and 11 of 16 evaluable patients with solid tumors had SD [78].

Another phase I dose-escalation trial evaluated BII021 in combination with trastuzumab in patients with HER2+ metastatic breast cancer. Primary endpoint was to evaluate the safety and tolerability of BII021 when administered orally twice weekly in combination with 6 mg/kg trastuzumab every 3 weeks. MTD was determined at 600 mg twice weekly. DLT were diarrhea and partial seizure that presented as aphasia. Other frequent adverse events included fatigue, nausea, dizziness, headache and rash among others. Of the 30 patients enrolled, 2 patients had confirmed PR by RECIST and 10 had SD. Three patients had metabolic PR (>25% decline in the SUV max) and 16 had SD on FDG-PET imaging [79].

Based on these data, phase II studies with this compound in combination with Aromasin were planned [80], however, BII021 is no longer listed on the Biogen Idec pipeline. Apparently, Biogen Idec no longer will develop BII021 as they are shifting focus away from oncology for strategic purposes, and plan to outlicense its further development [81].

2.2.2. MPC-3100

MPC-3100 (Table 1, entry 6) is being developed by Myrex, a company under the umbrella of Myriad Pharmaceuticals [82]. A phase I, modified Fibonacci, dose-escalation trial evaluated oral daily dose of MPC-3100 for 21 days in a 28-day cycle in patients with refractory or relapsed cancer. Single patient cohorts were studied for the first three dose levels (50, 100, 165 mg/m²). The first patient treated at 245 mg/m² developed grade 2 prerenal azotemia. Two additional patients were therefore treated in this cohort. One of these patients experienced a DLT of supraventricular tachycardia and respiratory failure after day 21 dose. Patients were enrolled at the 245 mg/m² cohort which was expanded after the development of the first DLT [83]. MPC-3100 recently completed the phase 1 study and results are awaited. Due to the poor solubility and bioavailability of MPC-3100, Myrex announced the planned introduction of a pro-drug, MPC-0767. The expected filing of an IND for the pro-drug candidate MPC-0767 is in the first quarter of 2012 [84].

2.2.3. Debio 0932 (CUDC-305)

Scientists at Curis replaced the N3 of the purine (Fig. 1, depicted in red on PU3) with a carbon to result in a purine-like derivative, Debio 0932 (CUDC-305) [85], which is being developed by Debiopharm (Table 1, entry 7) [86]. In April 2010, Debiopharm initiated the phase I trial that is designed to evaluate the MTD and safety of Debio 0932 in patients with advanced solid tumors or lymphoma. The first part of this study (phase Ia) is an open-label, dose-escalation, phase trial where the study drug will be administered orally (25–100 mg) in both daily and every other day regimens. A total of 80 patients are expected to be treated in the phase Ia portion. The phase Ib portion is an expansion cohort of certain solid tumors and/or lymphoma patients which will further assess the safety, PK and PD of this agent at a potential phase II dose level to make a preliminary assessment of anti-tumor activity. About 40 patients are expected to be treated on this phase Ib portion. The trial is currently ongoing and results are awaited [87].

2.2.4. PU-H71

The Chiosis group at Memorial Sloan-Kettering has discovered the Hsp90 inhibitor PU-H71 [88–90], which is being evaluated in a phase I clinical trial in patients with advanced solid tumors, lymphoma and myeloproliferative disorders under license to Samus Therapeutics (Table 1, entry 8). The primary objective is to assess the safety,

tolerability, MTD, and PK of PU-H71 in patients with advanced solid tumors and lymphoma when administered intravenously three times weekly for 2 weeks in a 21-day cycle. Secondary objectives are to assess anti-tumor activity as defined by response rate, CR, partial response (PR), stable disease, duration of response, and progression free survival (PFS), and to evaluate ^{124}I -PU-H71 as a non-invasive means to determine tumor PK and intratumor concentration in a selected group of patients with advanced malignancies. Exploratory objectives are to evaluate ^{124}I -PU-H71 as a non-invasive means to predict tumor dose–response relationships in a selected group of patients with advanced malignancies, to evaluate changes in tumor client proteins using optional tumor biopsies performed pre- and post-treatment, to evaluate *ex vivo* the sensitivity of the tumor to PU-H71 by performing optional tumor biopsies pre-treatment and to evaluate changes in client proteins in the circulating tumor cells pre- and post-treatment. This phase I trial began patient accrual in early August 2011.

This agent is also being studied at a different dosing schedule at the NCI in patients with advanced solid tumors and low-grade non-Hodgkin's lymphoma. Patients will receive PU-H71 intravenously weekly \times 2 weeks of 21-day cycle. This study will follow a modified accelerated titration design; the accelerated phase will end when one patient experiences a DLT or two patients experience grade 2 drug-related toxicities during the first cycle, after which the study will follow a standard 3 + 3 design. The primary objectives are to establish the safety, tolerability, MTD, recommended phase 2 dose, and PK of PU-H71 when administered on the above mentioned schedule. Secondary objectives are to perform pharmacodynamic (PD) studies to ascertain the effect of PU-H71 on Hsp90 client proteins in tumor tissue at the MTD, and on Hsp70 in tumor tissue, serum, and peripheral blood mononuclear cells at the MTD. PK and PD studies will be conducted during cycle 1. Up to 10 additional patients will be entered at the MTD to further define toxicity and perform PD studies at this dose. Planned accrual is up to 50 patients. This study is actively recruiting patients since June 2011 [91].

2.3. Resorcinol derivatives

RD is a macrocyclic lactone antibiotic first isolated from the fungus *Monosporium bonorden* in 1953 (Fig. 1) [92]. In addition to a resorcinol moiety (depicted in red in Fig. 1), RD also contains reactive epoxide and $\alpha,\beta,\gamma,\delta$ -unsaturated carbonyl groups. RD is not stable in serum and is devoid of *in vivo* activity, however, the resorcinol core is maintained in a number of molecules entering clinical trials including NVP-AUY922, KW-2478, and AT13387 as well as STA-9090 (Table 1, entry 9–13). While these molecules were not discovered through direct modification of RD they clearly resemble it by maintaining the resorcinol core as a critical element for binding.

2.3.1. Triazole derivatives STA-9090 (Ganetespib)

STA-9090 (Table 1, entry 9) is an unspecified novel resorcinol-containing triazole compound developed by Synta Pharmaceuticals and has been tested in multiple clinical trials in both advanced solid tumors and hematological malignancies.

Goldman et al. evaluated STA-9090 in a phase I trial in 35 patients with advanced solid tumors wherein they received this agent intravenously weekly for 3 weeks in a 28-day cycle. MTD was determined at 216 mg/m². DLT was fatigue, diarrhea and elevated amylase. Other common adverse events included abdominal pain, anemia, nausea, constipation and dyspnea. There was 1 PR in a patient with rectal cancer and several SD were noted in patients with gastrointestinal stromal tumor, non-small cell lung cancer and renal cell carcinoma [93]. Another phase I dose-escalation trial is evaluating twice weekly dosing (2–144 mg/m²) for 3 weeks in a 28-day cycle. Forty-nine patients have been treated so far. DLT was elevated liver enzymes. Of the evaluable patients, 1 PR was noted in a patient with metastatic

melanoma and there were 2 SD in patients with non-small cell lung cancer. STA-9090 was well tolerated up to 120 mg/m²; the 144 mg/m² dose cohort has been expanded and dose escalation is ongoing [94].

A safety and efficacy phase I/II study in patients with acute myeloid leukemia and other hematological malignancies evaluated weekly infusions of STA-9090 for 4 consecutive weeks per cycle at 3 dose levels (120–200 mg/m²). One patient had a DLT of elevated liver enzymes. There were no formal responses noted but one patient with refractory acute myeloid leukemia had SD and bone marrow blast reduction lasting 10 weeks. The recommended phase II dose has not been identified and accrual is ongoing [95]. Another multicenter, phase I trial evaluated STA-9090 administered intravenously twice weekly at doses ranging 14–110 mg/m² for 4 weeks. DLT included hyponatremia, hyperbilirubinemia, elevated liver enzymes and prolonged QTc. MTD has not been determined. Clinical responses included hematological responses in 2 patients with chronic myeloid leukemia (CML) and acute myeloid leukemia respectively. In addition, 4 patients with myelofibrosis had SD; 1 of these responses lasted 7 months and the patient went on to receive allogeneic stem cell transplant [96].

A phase II trial of STA-9090 was conducted in patients with non-small cell lung cancer. Patients received weekly infusions of STA-9090 at 200 mg/m² for 3 weeks in a 28-day cycle. Patients were enrolled based on their mutation status; cohort A: EGFR, cohort B: KRAS, cohort C: EGFR and KRAS wild type (WT) and cohort D: EGFR and KRAS WT with adenocarcinoma histology. Additional mutational analysis of *BRAF*, *PIK3CA*, *ERBB2* and *MET*, as well as FISH analysis for *ALK* translocation (*EML4*-*ALK* gene-rearrangement), were performed in a subset of patients. Study design also included cohort E which would allow patients with clinical benefit in cohorts A through D to receive weekly docetaxel therapy in addition to ganetespib after progression of disease. Primary endpoint was PFS at 16 weeks with expansion for that particular cohort. Common adverse events reported were fatigue, diarrhea, nausea, anorexia and dyspnea which were all manageable and easily reversible. Expansion criteria were achieved for cohort C, including 1 PR and 7 patients with SD > 16 weeks [97]. Updated results were presented at the 14th World Conference on lung cancer in July, 2011 in Amsterdam. It was noted that 8 patients in cohort C/D harbored the *EML4*-*ALK*-rearrangement gene (*ALK* + tumors). Six out of 8 (75%) patients were crizotinib naïve and had tumor shrinkage in the target lesions. Additionally, another patient with *ALK* + tumor with crizotinib-refractory disease had impressive tumor regressions in the lung and responded to single agent ganetespib. Eight of thirteen (62%) patients with KRAS mutation also had tumor shrinkage. One patient with mixed response (16% target lesion shrinkage and development of new lesions) rolled over to cohort E at 8 months. A phase IIb/III trial of ganetespib and docetaxel in second line advanced non-small cell lung cancer patients has been initiated [98].

Similar to the patients with non-small cell lung cancer, patients with gastrointestinal stromal tumor were also treated with STA-9090 at 200 mg/m² weekly for 3 weeks in a 28-day cycle. In this Simon's two stage study design, if >4/23 patients in stage 1 had a CBR > 16 weeks, then enrollment would continue to stage 2. Hsp90 client protein levels were analyzed in pre- and post-treatment tumor biopsies. Common adverse events noted were fatigue, diarrhea, nausea, vomiting and increased alkaline phosphatase. 12/23 evaluable patients had SD meeting the formal criteria to enroll to stage 2. However, paired tumor biopsies in 4 patients did not show prolonged inhibition of activated KIT or its downstream pathways. Accrual has been limited to patients with PDGFRA mutations to allow alternative schedules and combinations [99].

An open label phase II trial of STA-9090 is currently ongoing in patients with unselected metastatic breast cancer. STA-9090 is administered at the same dose and schedule used in patients with

non-small cell lung cancer and gastrointestinal stromal tumor. Primary endpoint is to determine overall RR using RECIST 1.1 [100].

2.3.2. Isoxazole derivatives NVP-AUY922/VER52296

Workman and colleagues from the Cancer Research UK Center for Cancer Therapeutics first identified the resorcinol containing pyrazole CCT018159 from a HTS of a library of 56,000 compounds capable of inhibiting the ATPase activity of yeast Hsp90 [101]. Using a structure-based approach, scientists at Vernalis and the Cancer Research UK Center for Cancer Therapeutics optimized this hit into the isoxazole NVP-AUY922/VER52296 (Table 1, entry 10) [102,103]. This compound is now being developed by Novartis and is currently in phase I/II clinical trials in patients with relapsed or refractory multiple myeloma and HER2+ and ER+ metastatic breast cancer.

Single agent NVP-AUY922 was administered intravenously weekly in 28-day cycle to patients with advanced solid tumors in a phase I study. A total of 96 patients were treated at doses ranging 2–70 mg/m². Endpoints also included PK and PD analyses. DLT was atrial flutter, diarrhea, fatigue, darkening of vision and anorexia. Other common adverse events included nausea (35%), vomiting (18%), and night blindness (20%). MTD was 70 mg/m². SD was reported in 16 patients and 9 patients had a partial metabolic response on FDG-PET [104]. This led to the phase II expansion in patients with HER2+ and ER+ metastatic breast cancer. Preliminary imaging and biomarker data from this phase II trial were reported in the 2011 Annual ASCO Scientific meeting. Patients with HER2+ and ER+ disease underwent FDG-PET with trastuzumab and bevacizumab respectively at baseline and during treatment. Two HER2+ patients had partial metabolic response and 1 of these patients had confirmed PR by RECIST [105].

A phase I/II study to determine the MTD of NVP-AUY922 alone and in combination with bortezomib, with or without dexamethasone, in patients with relapsed or refractory multiple myeloma has been completed and results are awaited [106].

Although the chemical structure of HSP990, an oral Hsp90 inhibitor, has not yet been reported, it is claimed to be a follow-up compound of NVP-AUY922. A phase I trial is evaluating HSP990 when administered either weekly or twice weekly in patient with advanced solid tumors. Endpoints include MTD, DLT, efficacy, PK and PD. The study is currently actively recruiting patients [107]. Another phase I trial is assessing the once weekly dose in adult Japanese and Korean patients with advanced solid tumors [108].

2.3.3. Other resorcinol derivatives

2.3.3.1. KW-2478. The novel resorcinol analog KW-2478 was discovered by Kyowa Hakko Kirin Pharma through “a unique lead optimization strategy, including microbial screening, X-ray crystallography, cell-based screening, and in vivo models” (Table 1, entry 12) [109]. A phase I safety, PK and PD study of KW-2478 were recently completed in patients with relapsed/refractory multiple myeloma, chronic lymphocytic leukemia or B-cell non-Hodgkin's lymphoma. KW-2478 (14–99 mg/m²) was administered intravenously daily on days 1–5 of a 14-day cycle. There were no DLTs observed up to 99 mg/m². Drug related toxicities included grade 1/2 hypertension in one patient and grade 3 QTc prolongation in another. The study is ongoing at a dose of 132 mg/m² and further dose-escalation is planned [110]. Another phase I/II trial is evaluating this agent in combination with bortezomib when administered on days 1, 4, 8, 11 of a 21-day cycle in patients with relapsed and refractory multiple myeloma [111].

2.3.3.2. AT-13387. AT13387 was discovered by scientists at Astex following optimization of a resorcinol-containing lead generated through a fragment-based drug discovery approach (Table 1, entry 13) [112,113]. Three phase I trials are evaluating this agent in various dosing schedules including weekly or twice weekly intravenous

infusions for 3 weeks in a 28-day cycle [114] and twice a week (2 days in a row) for the first 3 weeks in a 28-day cycle [115,116] in patients with advanced solid tumors. It is also being evaluated with or without Imatinib in a multicenter, randomized phase II study in patients with advanced gastrointestinal stromal tumor who have progressed following treatment with TKIs. AT-13387 will be administered IV weekly for 3 weeks of a 28-day cycle. Primary endpoint of this trial is to assess reduction/stabilization of tumor at 4 months using RECIST 1.1 criteria [117].

2.4. Dihydroindazolone derivatives

SNX-5422 is a pyrazole-containing Hsp90 inhibitor that was discovered by scientists at Serenex following optimization of a hit identified using an ATP-affinity column (Table 1, entry 14) [118]. This column was used to first load ATP-binding proteins, then was challenged with a library of structurally diverse 8000 compounds to identify molecules capable of displacing unspecified proteins from the column. Hsp90 was identified as the target of an initial hit compound. SNX-5422 is actually the glycine prodrug of SNX-2112 (Fig. 1). In May 2007, Serenex Inc started a phase I trial for SNX-5422 in patients with solid tumor and lymphoma. SNX-5422 (4–21.28 mg/m²) was administered orally every other day daily for 21 days in a 28-day cycle. Objectives were to determine MTD and PK of this agent. There were no DLTs observed among the 11 patients treated. Grade 1 toxicities included nausea and fatigue. The trial was enrolling at 21.28 mg/m² [119]. In March 2008, Pfizer Inc announced that it has entered into an agreement to acquire Serenex Inc [120]. Later, the development of SNX-5422 was discontinued based on reports of ocular toxicity and the potential for irreversible retinal damage [121].

2.5. Others

Other Hsp90 inhibitors include DS-2248 (Daiichi Sankyo, Inc; undergoing phase I evaluation) [122] and XL-888 (Exelixis; sponsor has decided to terminate the safety study of this agent) [123]. The structures for these two inhibitors have not been disclosed.

3. Assays for monitoring response to Hsp90 inhibition in clinic

Several clinical assays have been developed to assess Hsp90-target inhibition, mainly by measuring the levels of a number of Hsp90 onco-client proteins from samples obtained pre- and post-Hsp90 inhibitor administration. As mentioned, Hsp90 inhibition causes the proteasomal degradation of such client proteins [1,2,12]. Hsp70 induction, another read-out of Hsp90 inhibition established in several cancer models, was also evaluated.

Most such PD assays to date have been performed on peripheral blood mononuclear cells (PBMCs). Several potential biomarkers, such as CDK4, Raf-1, c-KIT reduction and Hsp70 induction were measured in these studies. While PD monitoring of PBMCs has provided a readily accessible and reproducible index of *in vivo* biologic activity of Hsp90 inhibitors in clinical trials, the current consensus from these studies is that drug effects in these cells do not predict tumor-specific activity [18,53,54].

This is not surprising in light of a body of evidence showing a higher sensitivity of cancer cells to Hsp90 inhibition when compared to normal cells, as well as preferential accumulation of these inhibitors in tumor cells rather than normal cells [124–126]. As a means of explanation, Kamal et al. proposed that Hsp90 in tumors exists entirely in multi-chaperone complexes of high affinity for certain small molecule inhibitors, whereas Hsp90 from normal tissues is in a latent, uncomplexed state of low inhibitor binding potential [123]. Recent work by Moulick et al. substantiates but also significantly extends this view by proposing that Hsp90 forms biochemically distinct

complexes in cancer cells [125]. In this view, a major fraction of cancer cell Hsp90 retains “housekeeping” chaperone functions similar to normal cells, whereas a functionally distinct Hsp90 pool enriched or expanded in cancer cells specifically interacts with oncogenic proteins required to maintain tumor cell survival. This Hsp90 fraction perhaps represents a cell stress specific form of chaperone complex, that is expanded and constitutively maintained in the tumor cell context, and that may execute functions necessary to maintain the malignant phenotype. Such roles are not only to regulate the folding of overexpressed (i.e. HER2), mutated (i.e. mB-Raf) or chimeric proteins (i.e. Bcr-Abl) [1–3], but also to facilitate scaffolding and complex formation of molecules involved in aberrantly activated signaling complexes (i.e. STAT5, BCL6) [88,125]. The abundance of this tumor Hsp90 species, which is not dictated by Hsp90 expression alone, is potentially predictive of the cell's sensitivity to Hsp90 inhibition [125].

Preclinical data also suggest that the levels of soluble IGFBP2 and HER2 extracellular domain levels [127] or serum Hsp70 might be a useful PD marker of drug response [128], however, the utility of these potential biomarkers has yet to be validated in clinical settings [129].

The use of biopsies to measure PD changes has also been attempted in an effort to assay target modulation [16], but remains limited because of the logistical and ethical issues associated with repeated invasive procedures. Studies in melanoma patients noted changes in biomarkers in patients that experienced prolonged stable disease when treated with 17-AAG [16] at the dose levels 320 and 450 mg/m²/week. When 17-AAG was administered i.v. once weekly \times 6 weeks at 450 mg/m² dose and schedule of 17-AAG, the effects of 17-AAG on Raf-1 kinase expression were short-lived, and no objective anti-melanoma responses were seen [25], suggesting collectively that at this dose and schedule, the tumor Hsp90 target was not optimally inhibited.

As an alternative to tumor biopsies, changes in the levels of tumor HER2 and VEGF levels are now being investigated non-invasively by PET using gallium-68 or zirconium-89 labeled antibodies [130–132]. This sensitive assay is able to monitor tumor receptor expression in real time following systemic Hsp90 inhibitor administration, and is currently incorporated in several ongoing clinical studies with Hsp90 inhibitors [132–134]. While results of these studies are awaited, it is evident that the use of these assays remains limited to tumors that express these markers.

PET with [¹⁸F]-FDG was also tested as an assay to measure early response to Hsp90 therapy. Preclinical studies in HER2 tumors suggested no changes in FDG-PET at 24 hour post Hsp90 inhibitor administration, in contrast to HER2 PET [135]. When incorporated into a phase I trial of IPI-504 in patients with metastatic TKI-resistant gastrointestinal stromal tumor who received the Hsp90 inhibitor on days 1, 4, 8 and 11 of a 21 day cycle, serial monitoring with ¹⁸FDG-PET/CT imaging at baseline, day 11, and day 21, showed a reduced FDG-PET signal. Reactivation of tumor FDG uptake correlated with planned breaks in drug administration, and the signal decreased again when treatment resumed [136]. On this trial only SD was noted and it remains unclear whether a complete FDG uptake inhibition rather than reduction in uptake is needed to result in CR and PR. Metabolic PR and SD have been reported with BIIB021 and NVP-AUY922 using FDG-PET imaging [79,104]. These data suggest that, at least in highly glycolytic tumors, FDG-PET may provide a useful assay to measure Hsp90 tumor inhibition over a longer drug administration time.

The Hsp90 inhibitor PU-H71 has an endogenous iodine atom (¹²⁷I), which can be conveniently replaced with the PET radionuclide ¹²⁴I to result in the imaging agent ¹²⁴I-PU-H71. Since the PET imaging agent is virtually identical to PU-H71, the use of this radioligand allows for assessing drug distribution by non-invasive PET imaging. A phase 0 trial of ¹²⁴I-PU-H71 which evaluates the radiation

dosimetry, metabolism and biodistribution of this PET agent is currently ongoing [137]. The purpose of this study is to see how PU-H71 accumulates and how long it lasts inside tumors. The results of the phase 0 trial will help plan how to use PU-H71 in the phase I setting; specifically it will allow optimization of the PET agent to assess tumor pharmacokinetics of PU-H71 in the phase I study. The PET assay will also be investigated in the phase I to evaluate tumor dose–response correlations by measuring PU-H71 tumor concentrations for up to 48 hours post co-infusion of PU-H71 with ¹²⁴I-PU-H71 [138]. Together, this may provide useful information on identifying tumors more likely to respond to PU-H71 therapy and in designing most optimal dose and schedules for the administration of the Hsp90 inhibitor.

4. Conclusion

Given their potential to degrade a number of different oncoproteins and affect multiple signaling pathways, Hsp90 inhibitors have been hypothesized to be active in a wide variety of cancers. To date, the greatest clinical activity as evidenced by objective tumor regressions, has been observed with the geldanamycin analog tanespimycin when given in combination with trastuzumab to patients with HER2+ metastatic breast cancer. Pre-clinically, HER2 is among the most sensitive client proteins to Hsp90 inhibition and this may account for the activity observed in this setting. Recently, objective tumor responses were reported with ganetespib in all tumors that harbored the ALK rearrangement. This further emphasizes the targeted approach of Hsp90 inhibition, in that responses are perhaps dependent upon addiction of the tumors to Hsp90 client oncoproteins. While these results validate Hsp90 inhibition as a relevant anti-cancer strategy, the full potential of these inhibitors to be active in a broader spectrum of tumor types has yet to be achieved. This differential response could be because we are unable to select patients who might best benefit from therapy and perhaps have not successfully optimized the dosing and scheduling of this class of agents.

As a class, Hsp90 inhibitors demonstrate rapid clearance from normal tissues and the blood compartment with prolonged retention in tumors, hence traditional serum pharmacokinetics may be insufficient to guide dosing and scheduling [4,88–90,103,125,139]. Additionally, the current drug development paradigm based on identifying maximum tolerated dose may not be applicable in the case of these inhibitors where target modulation is critical to producing their anti-tumor effects. In order to optimally deliver Hsp90 inhibitor therapy, evaluation of the effects of these agents at the level of the tumor (including client protein modulation) is essential. To this end, 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. Ultimately this investment into understanding the fundamentals of drug delivery and patient selection for these unique inhibitors will allow the next generation of Hsp90 targeted therapies to realize their full potential and promise as anti-cancer therapeutics.

Acknowledgement

G Chiosis is funded in part by Mr. William H. and Mrs. Alice Goodwin and the “Commonwealth Foundation for Cancer Research” and “The Experimental Therapeutics Center of Memorial Sloan-Kettering Cancer Center”, the Geoffrey Beene Cancer Research Center of MSKCC, Leukemia and Lymphoma Society, Breast Cancer Research Fund, the SPORE Pilot Award and Research & Therapeutics Program in Prostate Cancer, the Hirshberg Foundation for Pancreatic Cancer, the Byrne Fund, National Institutes of Health (1U01 AG032969-01A1, 1R01 CA155226-01, 1R21AI090501-01, 3P30CA008748), Department of Defense (R03-BC085588), Susan G Komen for the Cure

and the Institute for the Study of Aging and the Association for Frontotemporal Dementias (Grant #281207 AFTD). T Taldone discloses a grant support from the Department of Defense (PDF-BC093421), K Jhaveri from Conquer Cancer Foundation: Young Investigator Award and S Modi from ASCO Cancer Foundation/Breast Cancer Research Foundation: Advanced Clinical Research Award.

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